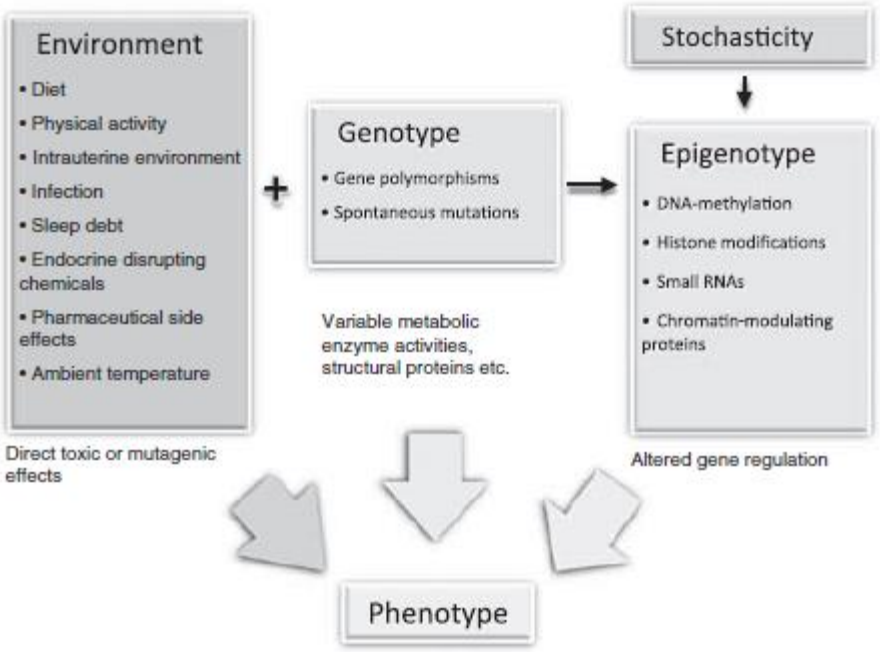
Επιγενετική και παχυσαρκία

Γιώργος Δεδούσης
Καθηγητής

- Cytosine methylation has primarily been studied in gene promoters because they are known to be central in regulation of transcription.
- Human studies of DNA methylation have typically been done using peripheral blood mononuclear cells. How well the DNA methylation pattern of peripheral blood mononuclear cells reflects that of other cell types at specific genomic sites is not definitively known.
- However, there are some information on DNA methylation patterns in various tissues in, for example, the Methylation Database (MethDB, www.methdb.de) and Next Generation Sequencing single-cytosine-resolution DNA METHylation DataBase (NGSmethDB, http://bioinfo2.ugr.es/NGSmethDB/gbrowse/hg18/). A list of additional resources on epigenomic research such as the human epigenome project initiative (www.epigenome.org)

Table 1. Summary of projects, programs and resources on epigenomic research[a]

Name	Aim	Website
The Human Epigenome Atlas	The Human Epigenome Atlas includes human reference epigenomes and the results of their integrative and comparative analyses. It is produced by the NIH Epigenomics Roadmap Consortium	*www.genboree.org/epigenomeatlas/index.rhtml*
NCBI Epigenomics Gateway	Curated, annotated and organized epigenetics-specific data selected from general-purpose archives, such as the Gene Expression Omnibus, and Sequence Read Archives	*www.ncbi.nlm.nih.gov/epigenomics*
Human Epigenome Project (HEP)	The HEP aims to identify, catalogue and interpret genome-wide DNA methylation patterns of all human genes in all major tissues	*www.epigenome.org*
RoadmapEpigenomics Project	The NIH Roadmap Epigenomics Mapping Consortium was launched with the goal of producing a public resource of human epigenomic data to catalyze basic biology and disease-oriented research	*www.roadmapepigenomics.org/* *www.epigenomebrowser.org/* *http://genomebrowser.wustl.edu/*
Epigenesys Network of Excellence (Epigenesys)	A new ambitious EC-funded research initiative on Epigenetics advancing towards systems biology	*www.epigenesys.eu/*
National Methylome 21 (NAME21)	NAME21 aims to generate a first comprehensive DNA methylation map on human chromosome 21 using bisulphite sequencing technologies	*http://biochem.jacobs-university.de/name21/*
Epigenetic Treatment of Neoplastic Disease (EPITRON)	EPIgenetic TReatment Of Neoplastic disease	*www.epitron.eu*
Highthroughput Epigenetic Regulatory Organization In Chromatin (HEROIC)	The HEROIC Project High-throughput Epigenetic Regulatory Organisation In Chromatin	*www.heroic-ip.eu*
AACR Human Epigenome Taskforce and Alliance for the Human Epigenome and Disease (AHEAD)	America Association Cancer Research (AACR) Human Epigenome Taskforce to implement the Alliance for the Human Epigenome and Disease (AHEAD) Project	*www.aacr.org/home/scientists/working-groups-task-forces/task-forces/human-epigenome-task-force.aspx*
The Common Fund's Epigenomics Program at NIH	The Epigenomics Program is part of the International Human Epigenome Consortium that aims to coordinate worldwide epigenome mapping and characterization efforts	*http://commonfund.nih.gov/epigenomics/*
International Human Epigenome Consortium (IHEC)	IHEC will coordinate epigenome mapping and characterisation worldwide to avoid redundant research effort, to implement high data quality standards, to coordinate data storage, management and analysis and to provide free access to the epigenomes produced	*http://ihec-epigenomes.org/index.html*



- Genetic predisposition to epigenetic changes also exists. This could be through DNA sequence variation in CG-sites, or small-RNA target sites in coding genes.
- Also, there may be DNA sequence variation in peptides in the epigenetic machinery, which could affect the outcome of the functional genome. Epigenetic influences that may alter gene expression are therefore sensitive not only to environment and chance, but also to the genetic make-up.
- The DNA sequence variation may hence affect both the stochastic and environmentally influenced variance in the epigenome

The obesity epidemic: from the environment to epigenetics – not simply a response to dietary manipulation in a thermoneutral environment

Michael E. Symonds*, Sylvain Sebert and Helen Budge

Early Life Nutrition Research Unit, Academic Division of Child Health, Nottingham Respiratory Medicine Biomedical Research Unit, School of Clinical Sciences, University Hospital, Nottingham, UK

Table 1 | Comparison of the macronutrient composition of a standard adult human diet with those used in nutritional studies involving primates and rodents.

Species	Control (or normal) diet (%)			High-fat:low–carbohydrate diet (%)			Reference
	Protein	Carbohydrate	Fat	Protein	Carbohydrate	Fat	
Human	17	50	31	–	–	–	Garriguet (2007)
Primate	18	72	11	16	31	52	McCurdy et al. (2009)
Rodent	21	71	6	15	55	15	Ainge et al. (2010)

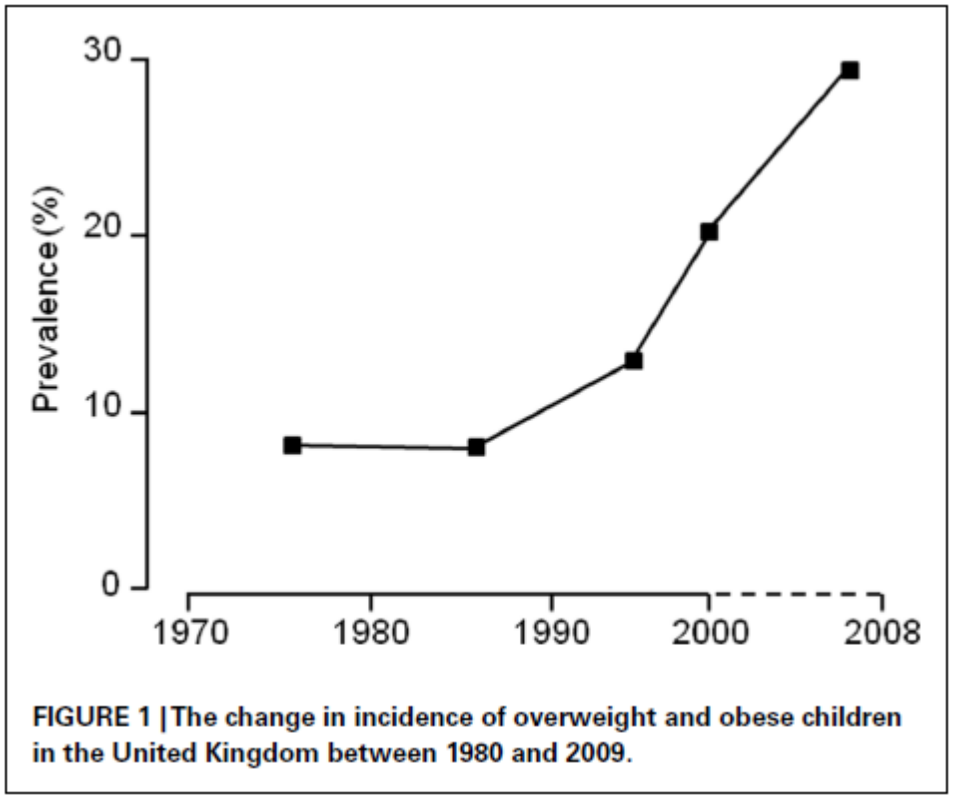


FIGURE 1 | The change in incidence of overweight and obese children in the United Kingdom between 1980 and 2009.

- Since the end of the Second World War, there has been a dramatic change in our lifestyles and many of the identified factors acting alone could be anticipated to promote obesity.
- The "step-wise" adaptations in lifestyles within the developed world have coincided with "rural flight," a substantial rise in the use of mechanical transportation a reduction in manual labor and altered working patterns with more shift work adding to disease risk.
- These, in turn, have a variety of social and health related effects even impacting upon reproductive success and birth weight

(Juneau and Potvin, 2010), (Puttonen et al., 2010; Szosland, 2011), (Ramsden, 2009).

Epigenetics Lights Up the Obesity Field

Amelia Marti[a,b] Jose Ordovas[b,c,d]

Obes Facts 2011;4:187–190

REVIEW

Epigenetic regulation in obesity

C Lavebratt[1], M Almgren[1,2] and TJ Ekström[2]

International Journal of Obesity (2012) 36, 757–765

- The methylation status is altered in the adipose tissue of inborn and diet-induced obese mice such that **hypermethylation of the –437 CpG** site upstream of the transcription start site of the PPARG2 gene was observed in omental adipose tissue of obese animals.
- Also rats raised in small litters become obese because of overfeeding, develop hyperglycemia and hyperinsulinemia, along with **hypermethylation** of the **proopiomelanocortin (POMC)** gene.
- CpG methylation has a role in the regulation of adipogenesis and glucose homeostasis.
- Tissue-specific level of CpG methylation is found for example in promoters of leptin, pro-opiomelanocortin (precursor for melanocyte-stimulating hormone (a-MSH), ACTH and b-endorphine) and insulin correlating to the corresponding tissue-specific expression levels.
- That changes in DNA methylation can occur postnatal can be exemplified by increase in CpG methylation of the key metabolic transcriptional regulator peroxisome proliferator-activated receptor-a (Ppar-a) by folate supplementation in young rodents.

Intervention trials on Mice

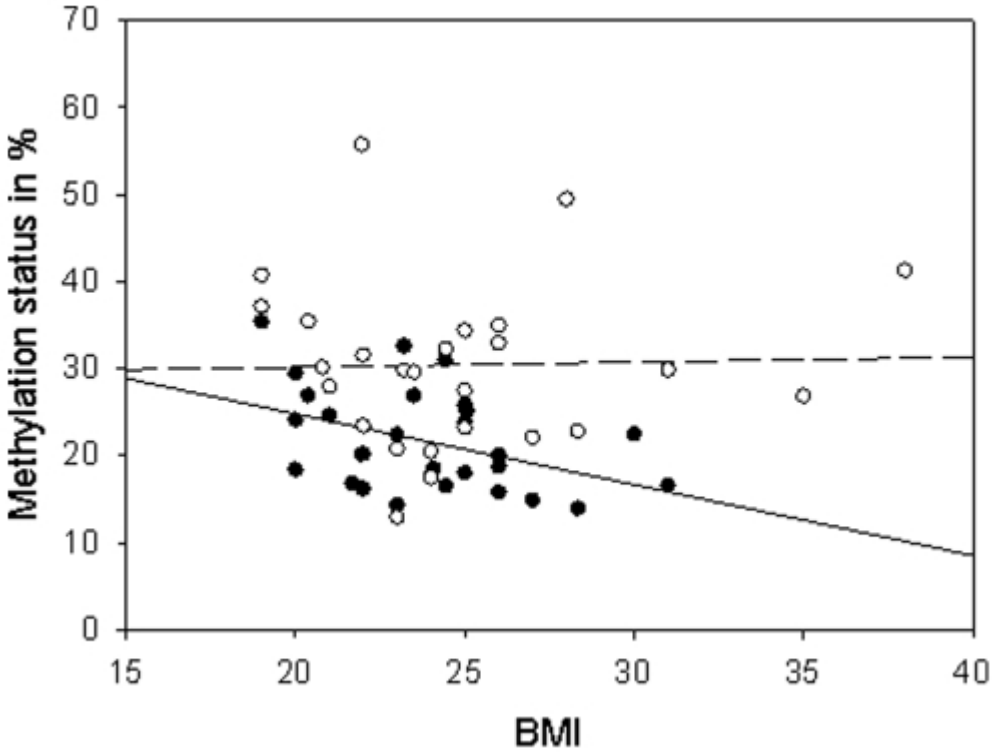
- Mice on high-fat diet developed hypomethylation in the **satiety-receptor melanocortin-4 receptor**, whereas neonatal overfeeding rats, and rats obese because of overfeeding in small litters, developed hypermethylation of the **satiety-mediator pro-opiomelanocortin** in hypothalamus.
- The promoter of **glucose transporter 4** was reported demethylated at adipocyte differentiation, and its methylation could inhibit nuclear factor binding to the promoter.
- Also the **Pparg2 promoter** was demethylated upon induction of adipocyte differentiation correlating with expression, and it was hypermethylated at one CpG in the visceral adipose tissue of genetically induced and diet-induced obese mice.
- Moreover, **FTO** that is genetically associated with obesity and type 2 diabetes, is a DNA **demethylase.**

J Appl Genet 2010; 51:193–197; J Physiol 2009; 587: 4963–4976.

The MCHR1 gene encodes a G protein-coupled receptor for melanin-concentrating hormone, a cyclic peptide that regulates a variety of functions in the mammalian brain, in particular feeding behavior. RT-PCR analysis showed that MCHR mRNA was widely expressed in brain tissues, pituitary, normal portions of adrenal glands (cortex and medulla), tumor tissues of adrenocortical tumors

CpG methylation level at a specific allele of a single-nucleotide polymorphism in melanin-concentrating hormone receptor 1 exon 1 was recently shown to associate to body mass index in peripheral blood mononuclear cell from young adults.

This may in part explain previous contradictory results on genetic association for this single-nucleotide polymorphism to obesity



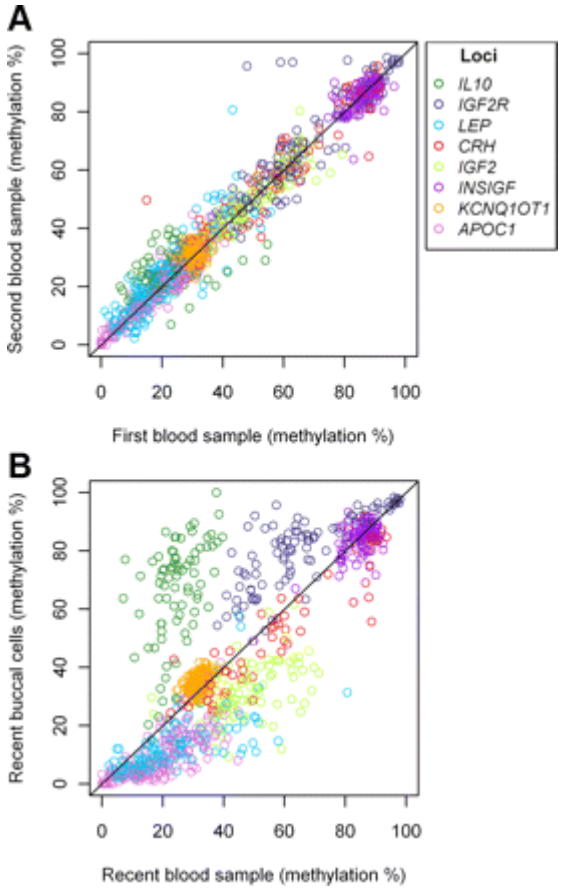
BMI-dependent DNA methylation levels at MCHR1

Allele specific methylation levels plotted against BMI of 39 individuals. The methylation status of the GT allele shows a significant negative correlation with BMI ($r = -0.814$, $P = 0.024$, Pearson correlation), whereas the methylation of the AC allele does not change with increasing BMI ($r = 0.057$, $P = 0.897$, Pearson correlation).

PLoS One 2011; 6: e17711

DNA methylation can change over time or not?

- There are reports suggesting that DNA methylation changes over time and mediates environmental effects on human disease.
- Other studies support that DNA methylation patterns are likely quite stable over time but variable between individuals as a result of inheritance.



Temporal stability and comparison between **blood** and **buccal cell** DNA methylation. A) CpG methylation in the first blood sample is plotted against methylation in the second, more recent, blood sample. B) CpG methylation in the recent blood DNA sample is plotted against methylation in the recent buccal swab DNA sample. Each dot represents 1 CpG unit of 1 individual in both DNA samples.

Nat Rev Genet 2007; 8: 253–262

FASEB J 2010; 24: 3135–3144

Personalized Epigenomic Signatures That Are Stable Over Time and Covary with Body Mass Index

Andrew P. Feinberg[1,2,*,†], **Rafael A. Irizarry**[1,3,*], **Delphine Fradin**[1,2,*,‡], **Martin J. Aryee**[1,4], **Peter Murakami**[1,2], **Thor Aspelund**[5,6], **Gudny Eiriksdottir**[5], **Tamara B. Harris**[7], **Lenore Launer**[7], **Vilmundur Gudnason**[5,6], and **M. Daniele Fallin**[1,8,†]

- Feinberg et al. have recently reported genome-wide human epigenetic data from **peripheral blood mononuclear cells** demonstrating 227 regions (VMRs) that showed variability in DNA methylation level between 74 elderly persons.
- **About 50%** of those VMRs remained stable within the person over 11 years whereas the rest of the VMRs changed over time.
- This supports that there are **stable epigenetic marks**, possibly governed by genetics, and **non-stable epigenetic marks** possibly influenced by environment.
- The stable VMRs can be used as an **epigenetic signature** for an individual, similar to genotype. These stable VMRs can be used as candidates for assessment of non-environmental DNA-methylation associations with disease or phenotype and hence predisposing variants of disease.

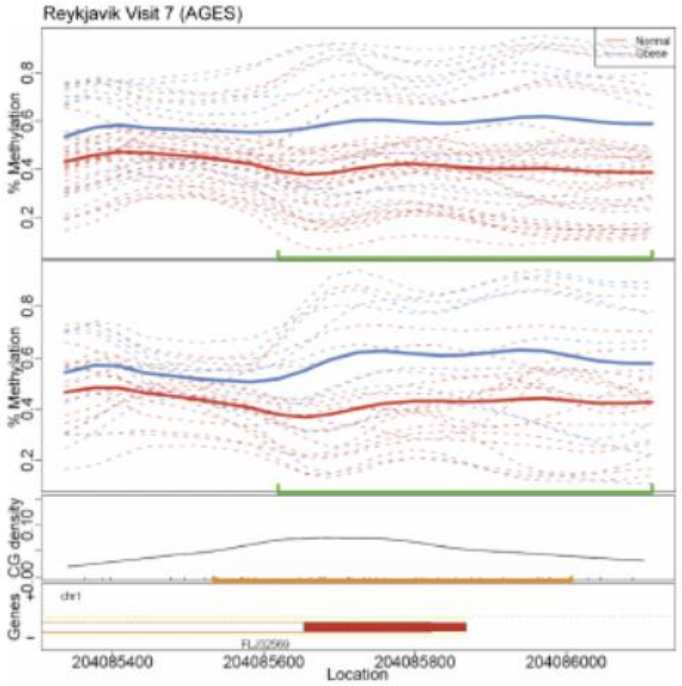


Fig. 3.
Methylation curves at the gene *PM20D1*. Methylation curves for visit 7 and visit 6 data. Dashed lines are individual methylation curves. Solid lines are average curves by obese (blue) and normal (red) groups. The green line indicates the boundaries of the VMR. CpG density is shown in the third panel, with CpG islands marked in orange. Gene location is shown in the bottom panel.

Sci Transl Med. 2010 September 15; 2(49): 49ra67.

- In fact, four of the stable epigenetic marks were correlated with body mass index at two time points 11 years apart.
- They resided in or nearby the genes **MMP9, PM20D1, PRKG1 and RFC5**. MMP9 and PM20D1 are metalloproteinases. MMPs (including MMP9) are known to be upregulated in human adipocytes, and have been associated with obesity in rodent models.
- **PRKG1**, a guanosine 30,50-monophosphate (cGMP)-dependent protein kinase is important in regulating **foraging behavior**, food acquisition and energy balance.

	Visit 6 (1991) $n = 48$	Visit 7 (2002–2005) $n = 64$
Age (years)	74.08 (3.49)	82.80 (3.45)
Sex (% male)	33	31
BMI (kg/m^2)	26.56 (3.81)	26.01 (4.10)
Type 2 diabetes (%)	8	11
Glucose (mM)	5.90 (0.59)	5.79 (0.88)
Coronary events (%)	10	14
Waist circumference (cm)	–	97.6 (10.9)
Fat (%)	–	29.31 (7.89)
Hemoglobin A1C (%)	–	5.69 (0.34)

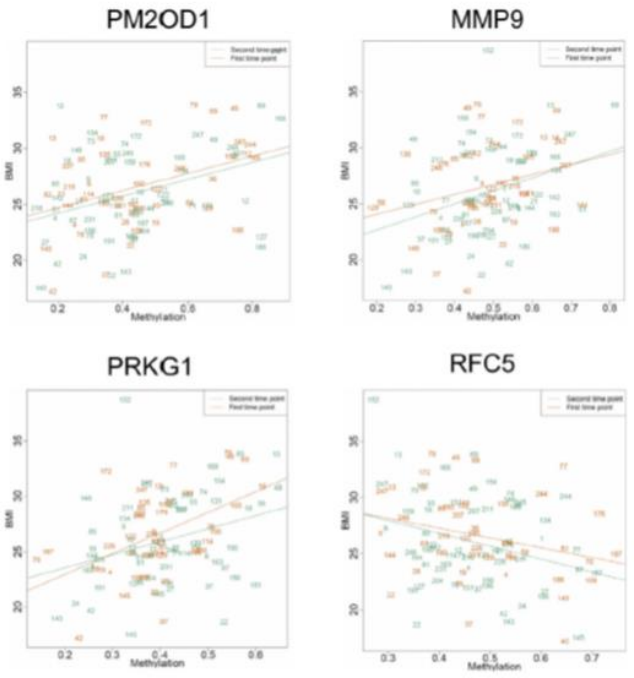
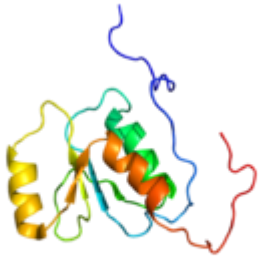
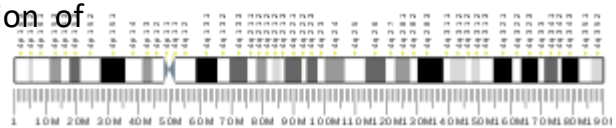


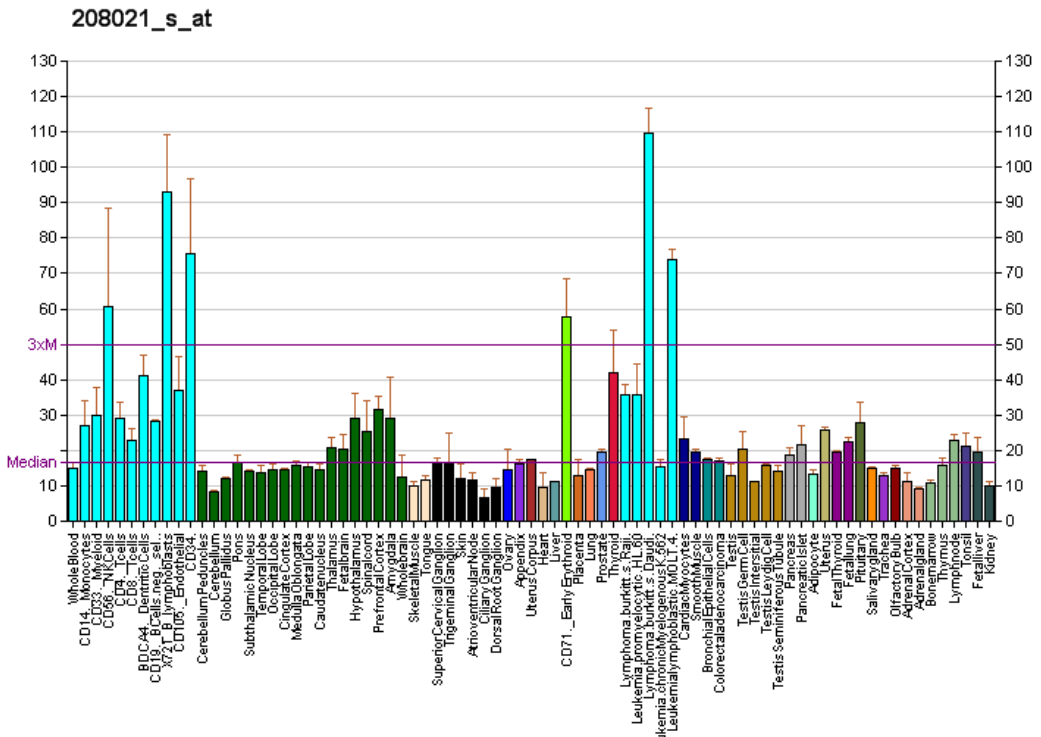
Fig. 4.
Correlations between methylation and BMI at six BMI-related VMRs. Points are individual IDs. Blue indicates visit 7; red indicates visit 6.

- The elongation of primed DNA templates by DNA polymerase delta and DNA polymerase epsilon requires the accessory proteins proliferating cell nuclear antigen (PCNA) and replication factor C (RFC). **RFC**, also named activator 1, is a protein complex consisting of five distinct subunits of 140, 40, 38, 37, and 36 kD. This gene encodes the 36 kD subunit. This subunit can interact with the C-terminal region of **PCNA**. It forms a core complex with the 38 and 40 kDa subunits. The core complex possesses DNA-dependent ATPase activity, which was found to be stimulated by PCNA in an in vitro system



- RFC5 is a metabolism-linked DNA replication complex loading protein, dysfunction of which leads to DNA repair defects.





Pregnancy and obesity in newborns

Epigenetic marks induced by the developmental environment are in agreement with the '**developmental plasticity**' hypothesis, that is, that the organism has an ability to undergo metabolic alterations during early development in order to match the phenotype to the anticipated future environment.

J Intern Med 2007; 261:461–471

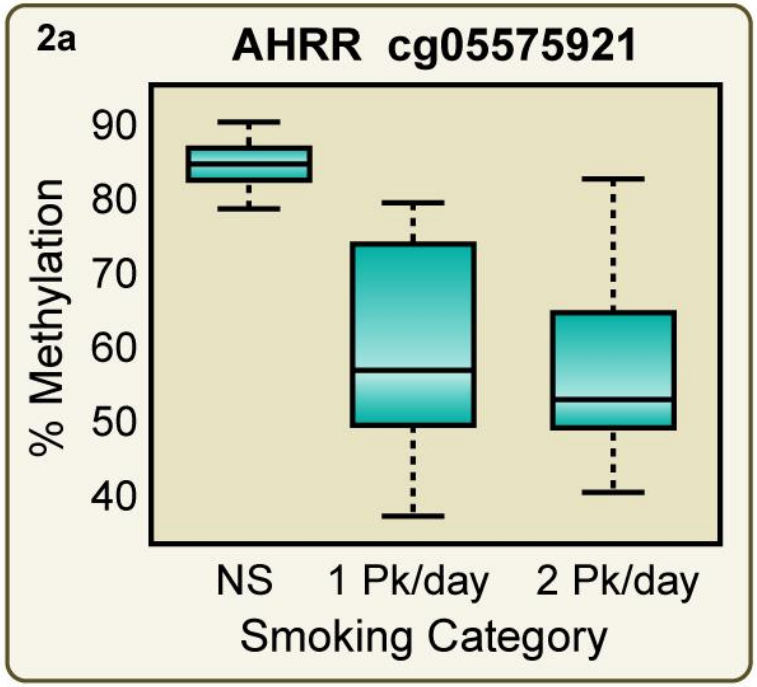
Large Maternal Weight Loss From Obesity Surgery Prevents Transmission of Obesity to Children Who Were Followed for 2 to 18 Years

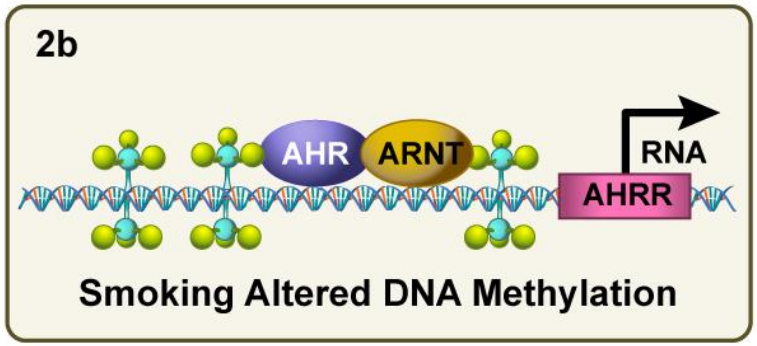
John G. Kral, Simon Biron, Serge Simard, Frédéric-Simon Hould, Stéfane Lebel, Simon Marceau, Picard Marceau

- To compare the prevalence of obesity in 172 children who were aged 2 to 18 years and born to 113 obese mothers (BMI: 31 ± 9 kg/m^2) with substantial weight loss after biliopancreatic bypass surgery with 45 same-age siblings who were born before maternal surgery (mothers' BMI: 48 ± 8 kg/m^2) and with current population standards.
- After maternal surgery, the prevalence of obesity in the offspring decreased by 52% and severe obesity by 45.1%, with no increase in the prevalence of underweight. The *z* score reduction in obesity was **gender specific**, with **boys** reducing from 1.4 ± 1.3 before to 0.57 ± 1.7 after maternal surgery, corrected for birth order. **The difference was not significant in girls** (0.8 ± 1.3 vs 0.8 ± 1.2). Among children of both genders who were aged 6 to 18 years of age and born after maternal surgery, the prevalence of overweight was reduced to population levels.
- **CONCLUSIONS.** Contrary to outcomes after intrauterine under- and overnutrition, the prevalence of overweight and obesity in children of mothers with large voluntary postsurgical weight loss was similar to that in the general population, with no increase in underweight. The results demonstrate the importance of potentially modifiable epigenetic factors in the cause of obesity.

Offspring DNA methylation of the aryl-hydrocarbon receptor repressor gene is associated with maternal BMI, gestational age, and birth weight

Heather H Burris, Andrea A Baccarelli, Hyang-Min Byun, Alejandra Cantoral, Allan C Just, Ivan Pantic, Maritsa Solano-Gonzalez, Katherine Svensson, Marcela Tamayo y Ortiz, Yan Zhao, Robert O Wright & Martha M Téllez-Rojo





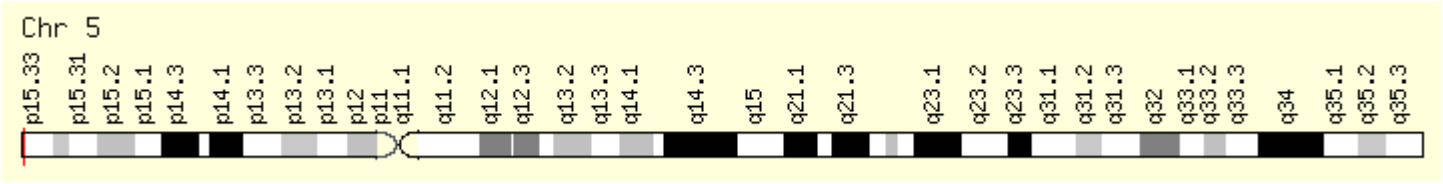
Epigenetics 10:10, 913–921; October 2015

Recent studies demonstrate that smoke exposure in adults and among fetuses exposed to maternal smoking is associated with altered leukocyte DNA methylation of the aryl-hydrocarbon receptor repressor (AHRR) gene, located on chromosome 5.

AHRR methylation is particularly interesting to study during pregnancy because the aryl-hydrocarbon receptor (AHR) is involved in metabolizing xenobiotics that might affect fetal development.

Table 1. Characteristics of PROGRESS birth cohort participants with umbilical cord DNA methylation from umbilical cord blood samples, n = 512, Mexico City, 2007–2010

	Mean	SD	Range
Maternal age (years)	27.8	5.5	18, 44
Second trimester measured BMI (kg/m^2)	26.9	4.2	17.4, 44.7
Pre-pregnancy self-reported BMI (kg/m^2)	25.2	4.2	16.0, 44.9
Gestational age (weeks)	38.8	1.8	24.4, 43.9
Birth weight (kg)	3.067	0.489	0.625, 4.625
Fenton BWT/GA Z score	−0.46	0.95	−5.72, 3.15
	n	%	
Maternal education			
Less than 12 years	203	39.7	
12 years	169	33.0	
More than 12 years	140	27.3	
Multiparous	279	54.7	
Household smoke exposure	239	46.8	
Male infant	282	55.2	



Mean and standard deviation (SD) methylation values (%5-methylcytosine) for the 3 AHRR CpG sites were 58.9 (7.8), 83.8 (6.2), and 64.3 (8.3) and were correlated with one another

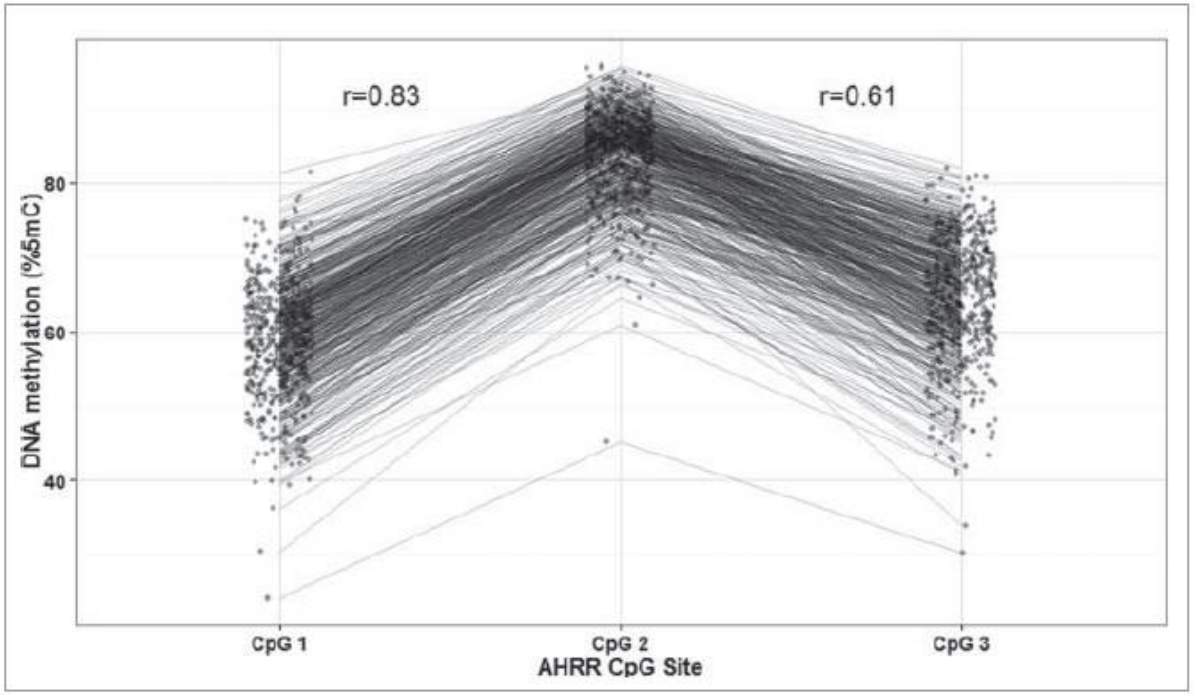
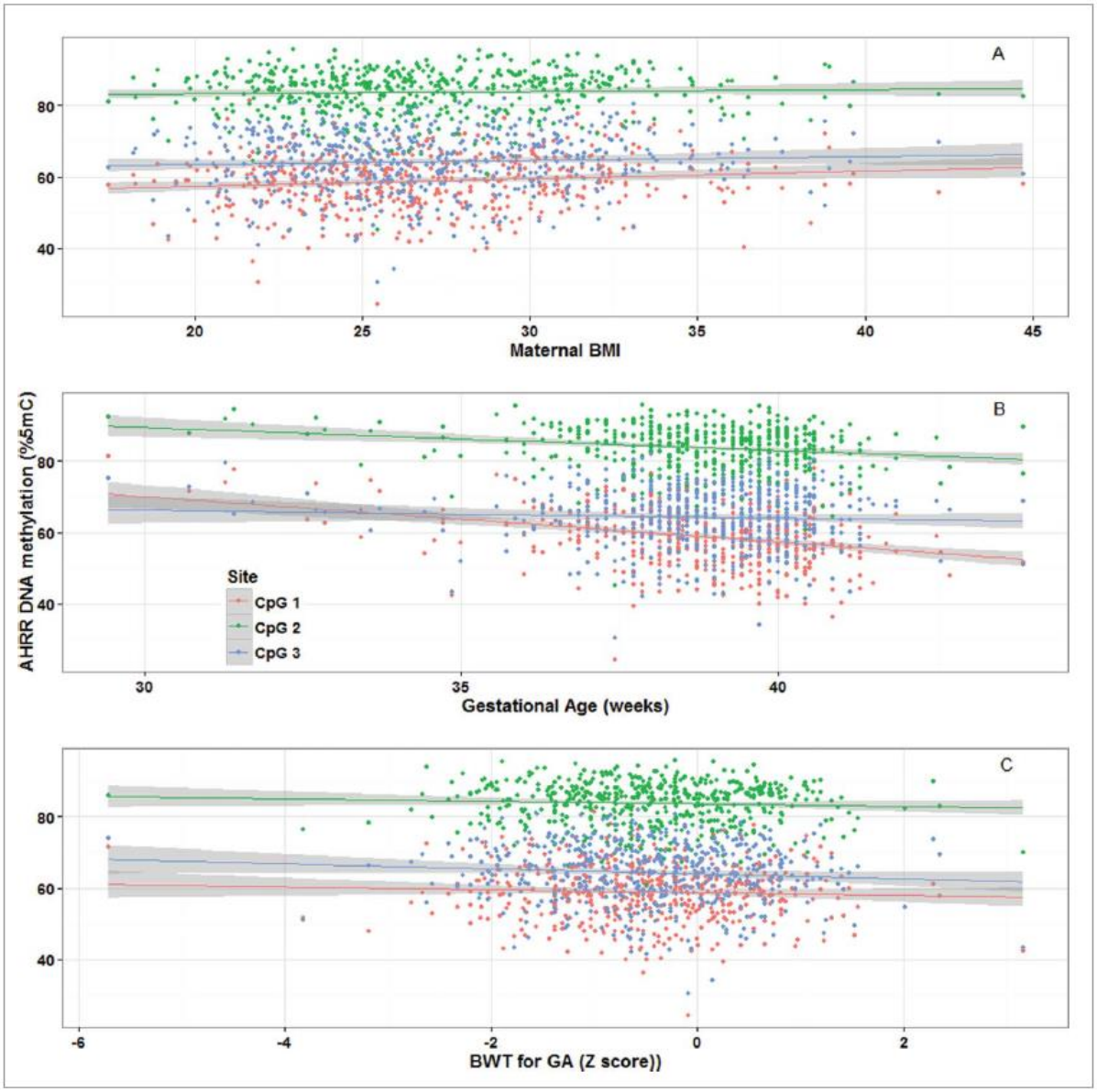


Figure 1. *Aryl-hydrocarbon receptor repressor* (*AHRR*) DNA methylation (%5-methylcytocines) at 3 CpG sites in cord blood DNA and their correlations, PROGRESS birth cohort, Mexico City, n = 512.

Table 2. Associations of offspring *AHRR* DNA methylation with maternal and infant characteristics, PROGRESS birth cohort, Mexico City, 2007–2010

	Single variable models[1]			Multivariable-adjusted model[2]		
	β (%5mC)	95% CI	*P* value	β (%5mC)	95% CI	*P* value
Maternal characteristics						
Maternal age (per year)	−0.02	(−0.09, 0.05)	0.5	−0.02	(−0.09, 0.05)	0.5
Maternal BMI (per kg/m^2)	0.13	(0.05, 0.21)	0.002	0.14	(0.06, 0.22)	0.0009[3]
Maternal education <12 years (vs. 12)	0.89	(0.00, 1.78)	0.05	0.77	(−0.09, 0.05)	0.08
Maternal education >12 years (vs. 12)	−0.12	(−1.10, 0.85)	0.8	−0.20	(−1.16, 0.75)	0.7
Multiparous (vs. primiparous)	−0.14	(−0.88, 0.61)	0.7	−0.33	(−1.09, 0.43)	0.4
Household smoke exposure (vs. no)	0.42	(−0.32, 1.2)	0.3	0.36	(−0.37, 1.09)	0.3
Infant characteristics						
Gestational age (per week)	−0.43	(−0.78, −0.08)	0.02	−0.86	(−1.06, −0.65)	<0.0001[3]
Birth weight-for-gestational age (per SD)	−0.49	(−0.88, −0.10)	0.01	−0.97	(−1.26, −0.58)	<0.0001[3]
Male (vs. female)	0.87	(0.12, 1.6)	0.02	0.48	(−0.25, 1.21)	0.2

Bivariate analysis revealed that maternal BMI and education <12 years vs. 12 years, infant sex, gestational age, and birth weight-for-gestational age were all associated with AHRR DNA methylation



Plots of AHRR DNA methylation with maternal BMI, gestational age, and birth weight-for gestational age reveal that the associations were similar among all 3 CpG sites, although weakest for CpG3

Figure 2. *Aryl-hydrocarbon receptor repressor* (*AHRR*) DNA methylation (%5-methylcytocine) and (**A**) maternal BMI; (**B**) infant gestational age; and (**C**) birth weight-for gestational age, PROGRESS birth cohort, Mexico City, n = 512.

Table 3. Multivariable associations of offspring *aryl-hydrocarbon receptor repressor* (*AHRR*) DNA methylation with categories of maternal BMI, preterm vs. term birth, and categories of fetal growth, PROGRESS birth cohort, Mexico City, n = 507

	β (***AHRR*** **DNA methylation, %5mC)**	**95% CI**	***P*** **value**
Model 1			
Overweight vs. normal weight	0.91	(0.08, 1.74)	0.03
Obese vs. normal weight	2.14	(1.01, 3.23)	0.0001
Model 2			
Preterm vs. full term	3.15	(1.97, 4.33)	<0.0001
Model 3			
SGA vs. AGA	1.11	(0.20, 2.02)	0.02
LGA vs. AGA	−3.72	(−6.20, −1.23)	0.003

They found that infants born to obese and overweight mothers had higher AHRR DNA methylation (**2.1**%) and (**0.9**%), respectively, vs. normal weight. Preterm (vs. full term) infants had higher AHRR DNA methylation (**3.1**%). LGA (vs. AGA) infants had lower AHRR DNA methylation (**3.7%)**.

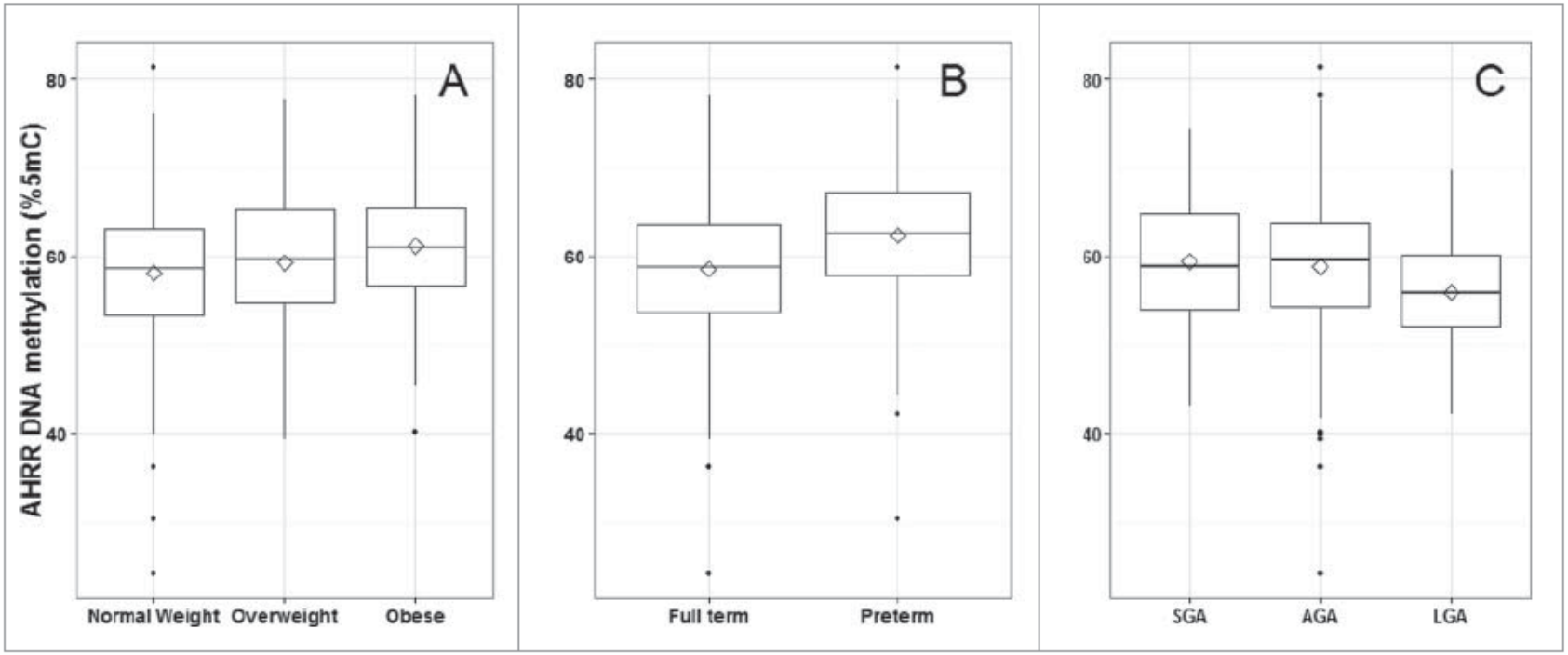


Figure 3. Distributions of *aryl-hydrocarbon receptor repressor* (*AHRR*) CpG 1 DNA methylation (%5-methylcytocines) among categories of (**A**) maternal BMI; (**B**) full-term vs. preterm births; and (**C**) birth weight-for-gestational age, PROGRESS birth cohort, Mexico City, n = 512. SGA, small-for-gestational age; AGA, appropriate-for-gestational age; LGA, large-for-gestational age.

Representative plots of AHRR CpG1 with BMI categories (normal weight, overweight, and obese), preterm vs. full term birth and SGA, AGA, and LGA demonstrate that the direction of the associations is the same whether continuous or categorical variables are used to predict AHRR DNA methylation

Conclusion

In this prospective birth cohort, they found significant associations of maternal BMI, infant gestational age, and birth weight-for-gestational age with offspring AHRR DNA methylation in umbilical cord blood DNA.

Future work to determine the long-term consequences of AHRR DNA methylation later in childhood is warranted.

Paternal obesity is associated with *IGF2* hypomethylation in newborns: results from a Newborn Epigenetics Study (NEST) cohort

Adelheid Soubry[1*], Joellen M Schildkraut[1,2], Amy Murtha[3], Frances Wang[1], Zhiqing Huang[4], Autumn Bernal[5], Joanne Kurtzberg[6], Randy L Jirtle[5], Susan K Murphy[4] and Cathrine Hoyo[7]

- Data from epidemiological and animal model studies suggest that **nutrition during pregnancy** may affect the health status of subsequent generations.
- These transgenerational effects are now being explained by disruptions at the level of the **epigenetic machinery**.
- Besides in vitro environmental exposures, the possible impact on the reprogramming of methylation profiles at imprinted genes at a much earlier time point, such as **during spermatogenesis or oogenesis**, has not previously been considered.
- In this study, the aim was to determine associations between **preconceptional obesity** and **DNA methylation profiles in the offspring**, particularly at the differentially methylated regions **(DMRs)** of the imprinted **Insulin-like Growth Factor 2 (IGF2) gene**.

Soubry *et al. BMC Medicine* 2013, **11**:29

Table 1 Parental and newborn characteristics

NEST - Newborn Epigenetics Study Cohort 2005 to 2006		n	%
BMI mother:	BMI <30 (not obese)	**59**	**67.8**
	BMI ≥30 (obese)	**28**	**32.2**
BMI father:	BMI <30 (not obese)	**63**	**79.7**
	BMI ≥30 (obese)	**16**	**20.3**
Marital status:	Living with partner	**72**	**74.2**
	Single	**25**	**25.8**
Education:	Low (no college degree)	**57**	**58.8**
	High (at least college degree)	**40**	**41.2**
Race:	African American	**38**	**38.8**
	Caucasian	**56**	**57.1**
	Other or not specified	**4**	**4.08**
Maternal age:	<30 years	**56**	**57.1**
	≥30 years	**42**	**42.9**
Smoking:	Mother never smoked	**45**	**48.4**
	Quit smoking when pregnant	**26**	**27.9**
	Smoked during pregnancy	**22**	**23.7**
Gestation time:	Preterm (<37 weeks)	**10**	**10.3**
	Normal (≥37 weeks)	**87**	**86.7**
Birth weight:	<2.5 kg	**16**	**16.5**
	≥2.5 kg	**81**	**83.5**
Baby gender:	Male	**48**	**49.5**
	Female	**49**	**50.5**

This sub-cohort includes all NEST families from whom babies were born at Duke University Hospital between July 2005 and November 2006. Characteristics of mothers, fathers and newborns are shown for the 98 participants when data were not missing.

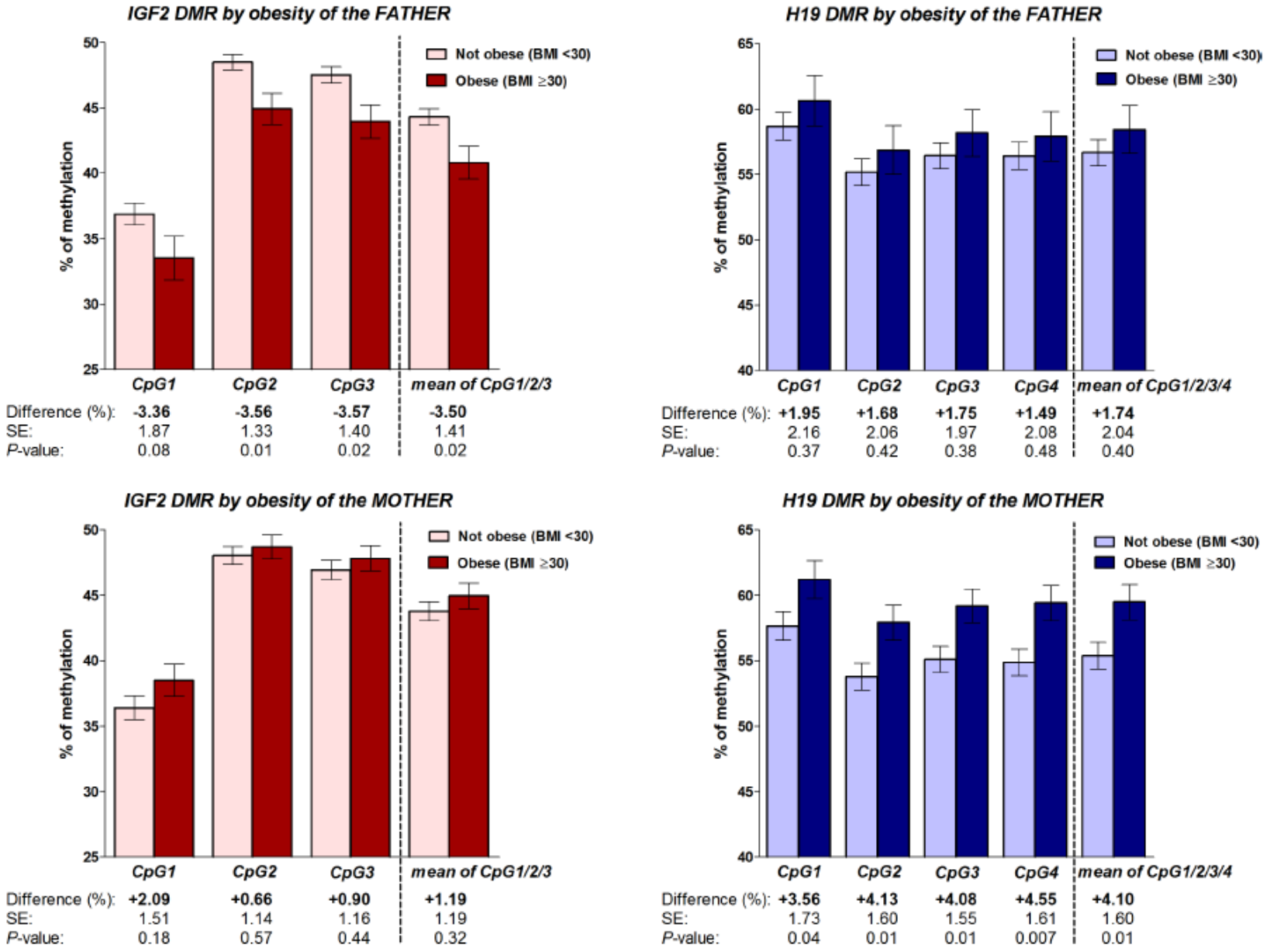


Figure 1 Methylation at the *IGF2* and *H19* DMRs in the offspring by parental obesity. The graphs represent the mean estimated methylation values of 69 newborns at the *IGF2* DMR, and 70 newborns at the *H19* DMR. The *IGF2* DMR results are based on 14 obese fathers and 25 obese mothers; the results at the *H19* DMR are based on 15 obese fathers and 23 obese mothers. For each exposure the differences of the least square means of methylation percentages are shown at each CpG site (bold), as well as standard errors (SE) and *P*-values. Bars represent standard errors.

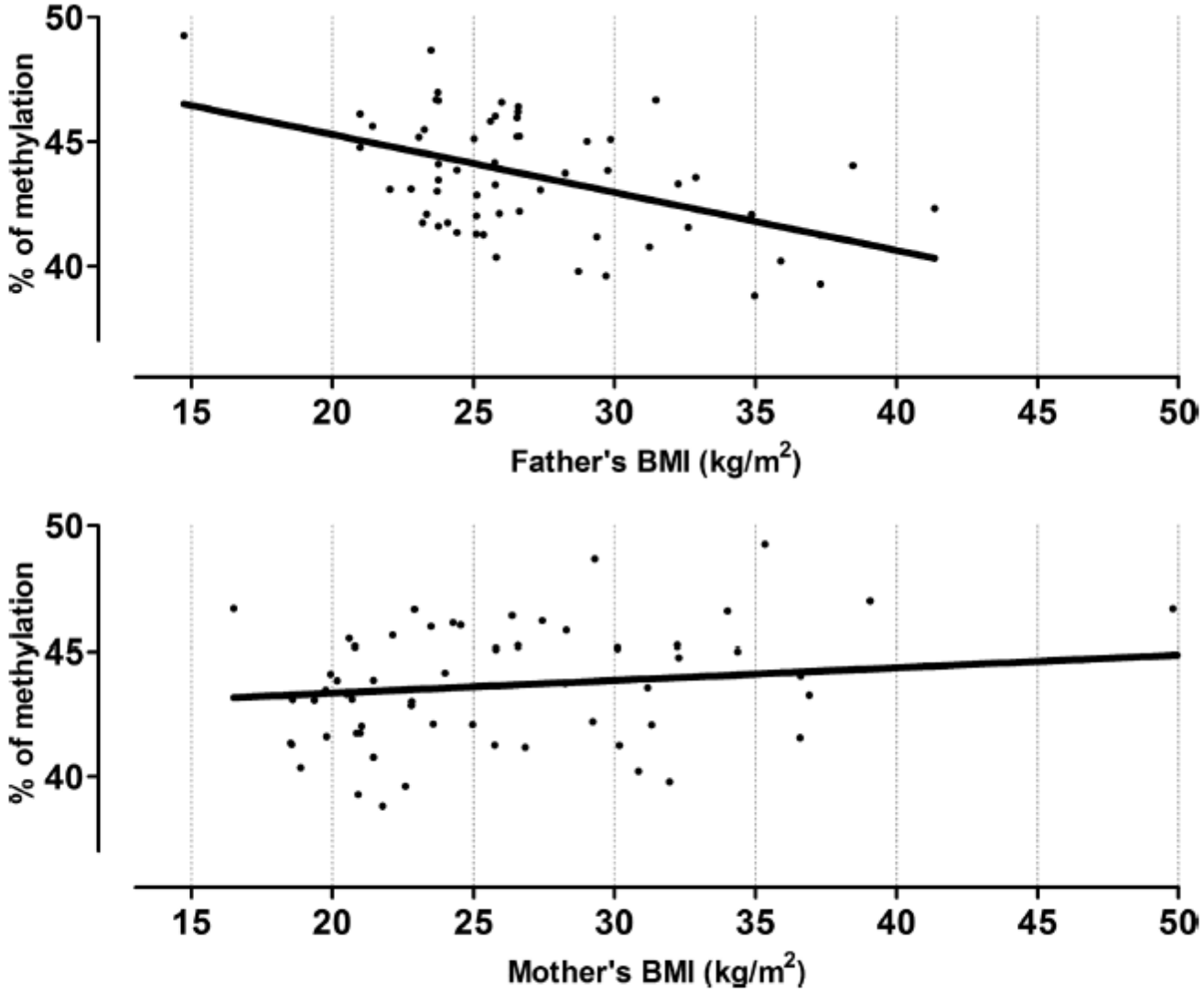


Figure 2 Offspring's mean methylation % at the *IGF2* DMR by BMI of the parents. The predicted methylation means at *IGF2* DMR are plotted by BMI of the father (upper graph), and BMI of the mother (lower graph); adjusted for maternal age, smoking status, BMI of the other parent, the newborn's birth weight and gender.

Table 2 Linear Regression Models: methylation at the *IGF2* and *H19* DMRs in relation to parental obesity

Linear regression models		*IGF2* DMR			*H19* DMR		
	Obesity of:	**β**	SE	***P***	**β**	SE	***P***
Model 1	Father	**-3.83**	1.48	0.01	**+3.09**	1.64	0.07
Model 2	Mother	**+2.38**	1.30	0.08	**+2.80**	1.38	0.05
Model 3	Father	**-5.28**	1.62	0.003	**+2.55**	1.82	0.17
	Mother	**+3.08**	1.48	0.05	**+1.05**	1.70	0.54

Obesity was defined as BMI ≥30 kg/m^2. All models were adjusted for maternal age and smoking status, as well as by the newborn's birth weight and gender. Models 1 and 2 include either maternal or paternal obesity. Model 3 includes both maternal and paternal obesity.

Conclusions

- They found a significant decrease in methylation among newborns of obese fathers at the IGF2 DMR in DNA extracted from cord blood leucocytes.
- Examining possible associations between paternal or maternal obesity and birth weight but detected no associations.
- Hypomethylation at the IGF2 DMR has been associated with an increased risk of developing cancers, such as Wilms' tumor, colorectal cancer and ovarian cancer.
- Analyses of adults born to mothers exposed to poor nutrition during the Dutch famine indicated a 5% decrease in methylation at the IGF2 DMR compared to the same sex non-exposed siblings.
- Furthermore, not only do exposures during early gestation cause harmful health outcomes, but famine prior to conception has also been associated with poor health.
- Analysis of the Framingham Heart Study indicates that early-onset paternal obesity, and not maternal obesity, increases the odds of aberrant serum levels of the metabolic biomarker ALT (alanine transaminase) in the offspring.
- Male mice whose mothers were exposed to a high-fat diet were not only obese, insulin insensitive and diabetic, they were also capable of passing part of this phenotype to the next generation, suggesting an underlying epigenetic mechanism transmitted through germ cells

Conclusions II

- These results suggest that lifestyle factors of parents may be indirectly transmitted to the next generation via epigenetic mechanisms.
- One of the measurable lifestyle parameters, obesity of the father, is associated with hypomethylation at the IGF2 DMR in the offspring.
- The hypothesis is that incomplete or unstable establishment of methylation at the IGF2 DMR during gametogenesis.
- As a result, exposures to adverse lifestyle factors or poor/ over-nutrition during spermatogenesis may affect the reprogramming of methylation profiles at imprinted genes.

The FASEB Journal • Research Communication

A dual epigenomic approach for the search of obesity biomarkers: DNA methylation in relation to diet-induced weight loss

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Twenty-five overweight or obese men participated in an 8-wk caloric restriction intervention. DNA was isolated from peripheral blood mononuclear cells and treated with bisulfite. The basal and endpoint epigenetic differences between high and low responders were analyzed by methylation microarray, which was also useful in comparing epigenetic changes due to the nutrition intervention.

The main goals in this study

First, the description of differences between low and high responders to caloric restriction in the DNA methylation patterns of several genes. This study may allow the identification of epigenetic marks that could be used as predictive markers of weight loss in the design of personalized obesity prevention and management.

Second, the description of the effects of a hypocaloric diet treatment on the DNA methylation levels of different genes related to adiposity, inflammation, and weight regulation. This study would help to search new mechanisms of metabolic reprogramming owing to energydeprivation.

And third, the study of the differences between the DNA methylation patterns of high and low responders to the hypocaloric diet at the endpoint of the treatment, in order to associate them with the metabolic features of each subject.

TABLE 2. *Anthropometric and biochemical differences between high and low responders to the diet before intervention (baseline) and variation due to the weight-loss program*

Parameter	Baseline		Intervention-induced variation (%)	
	High responders, $n = 6$	Low responders, $n = 6$	High responders, $n = 5$	Low responders, $n = 5$
BW (kg)	96.7 ± 2.7	93.8 ± 4.2	−12.4 ± 0.6	−3.6 ± 0.5***
BMI (kg/m^2)	31.0 ± 0.7	30.2 ± 0.6	−12.6 ± 0.5	−3.6 ± 0.4***
Fat mass (kg)	26.7 ± 1.3	26.6 ± 2.0	−24.5 ± 1.2	−6.3 ± 1.8***
WC (cm)	102 ± 3	100 ± 2	−10.2 ± 0.6	−4.4 ± 0.8***
SBP (mmHg)	127 ± 5	131 ± 2	−3.2 ± 2.1	−4.9 ± 1.5
DBP (mmHg)	67 ± 3	77 ± 6	−3.8 ± 3.3	−17.0 ± 5.8*
Total cholesterol (mg/dl)	196 ± 21	237 ± 22	−24.9 ± 5.2	−1.2 ± 4.2***
HDL cholesterol (mg/dl)	47 ± 3	46 ± 2	−15.1 ± 5.1	−0.5 ± 6.6
LDL cholesterol (mg/dl)	127 ± 19	168 ± 17	−28.4 ± 7.9	−1.8 ± 4.2*
Triglycerides (mg/dl)	108 ± 17	117 ± 20	−23.0 ± 7.5	8.2 ± 17.9
Glucose (mg/dl)	92 ± 5	95 ± 3	3.0 ± 5.9	10.8 ± 3.4
Insulin (μU/ml)	17.9 ± 5.6	11.2 ± 1.8	−46.1 ± 21.2	−19.4 ± 6.7
HOMA	4.1 ± 1.4	2.6 ± 0.4	−38.6 ± 29.3	−10.3 ± 9.4
Leptin (ng/ml)	13.2 ± 2.9	13.8 ± 2.1	−66.2 ± 3.1	−21.8 ± 5.5***
Adiponectin (μg/ml)	8.6 ± 1.6	7.5 ± 0.7	−7.2 ± 11.2	11.9 ± 13.1***
Ghrelin (pg/ml)	982 ± 120	941 ± 55	6.6 ± 7.1	7.4 ± 5.8
PAI-1 (ng/ml)	172 ± 16	139 ± 19	−10.4 ± 12.0	−11.0 ± 10.2
IL-6 (pg/ml)	1.3 ± 0.1	3.1 ± 1.0	9.1 ± 21.1	−27.3 ± 20.8
TNF-α (pg/ml)	0.9 ± 0.2	1.5 ± 0.3	2.9 ± 35.1	−21.2 ± 14.5
Plasma MDA (μM)	2.1 ± 0.4	2.1 ± 0.3	−9.2 ± 7.5	2.0 ± 3.4

Values are means ± SE. Differences are nonsignificant except as noted; calculated by Mann-Whitney U test. *$P < 0.05$, ***$P < 0.001$ *vs.* high responders.

8-wk energy-restricted diet treatment with a 30% energy reduction

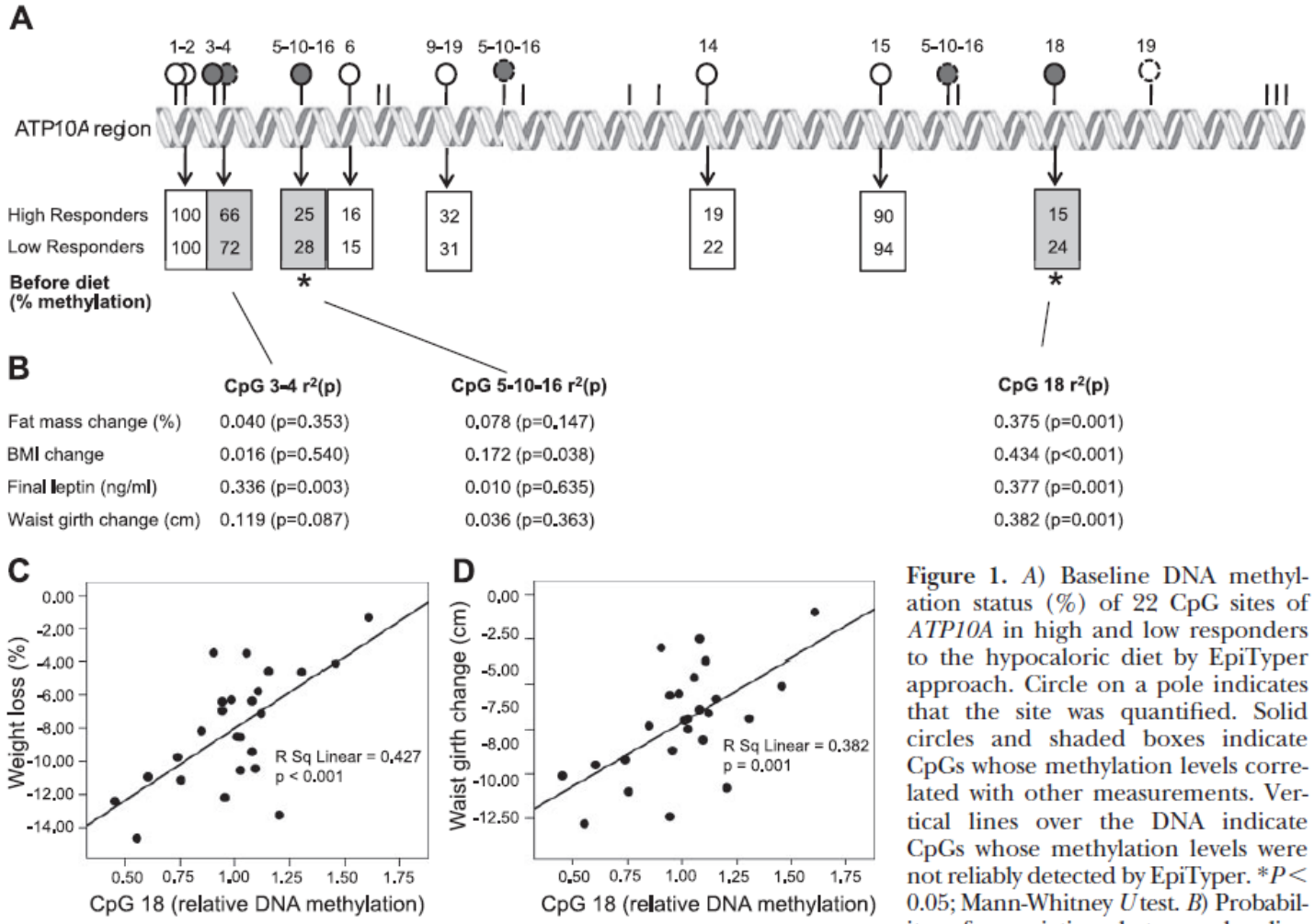


Figure 1. *A*) Baseline DNA methylation status (%) of 22 CpG sites of *ATP10A* in high and low responders to the hypocaloric diet by EpiTyper approach. Circle on a pole indicates that the site was quantified. Solid circles and shaded boxes indicate CpGs whose methylation levels correlated with other measurements. Vertical lines over the DNA indicate CpGs whose methylation levels were not reliably detected by EpiTyper. $*P<0.05$; Mann-Whitney *U* test. *B*) Probability of association between baseline methylation levels of the indicated CpGs and the intervention-induced changes in different anthropometric and metabolic measurements (Pearson's correlation test). *C*) Correlation analysis between baseline methylation levels of one CpG from *ATP10A* (CpG18) and weight loss during the intervention period (Pearson's correlation test). *D*) Correlation analysis between baseline methylation levels of CpG18 and WC variation during the intervention period (Pearson's correlation test).

ATP10A encodes an aminophospholipid translocase that transports phosphatidylserine and phosphatidylethanolamine from one side of a bilayer to another It is a type IV P-type ATPase related to lipid trafficking and maintenance of the phospholipid asymmetry and fluidity of the plasma membrane and seems to be involved in modulating body fat

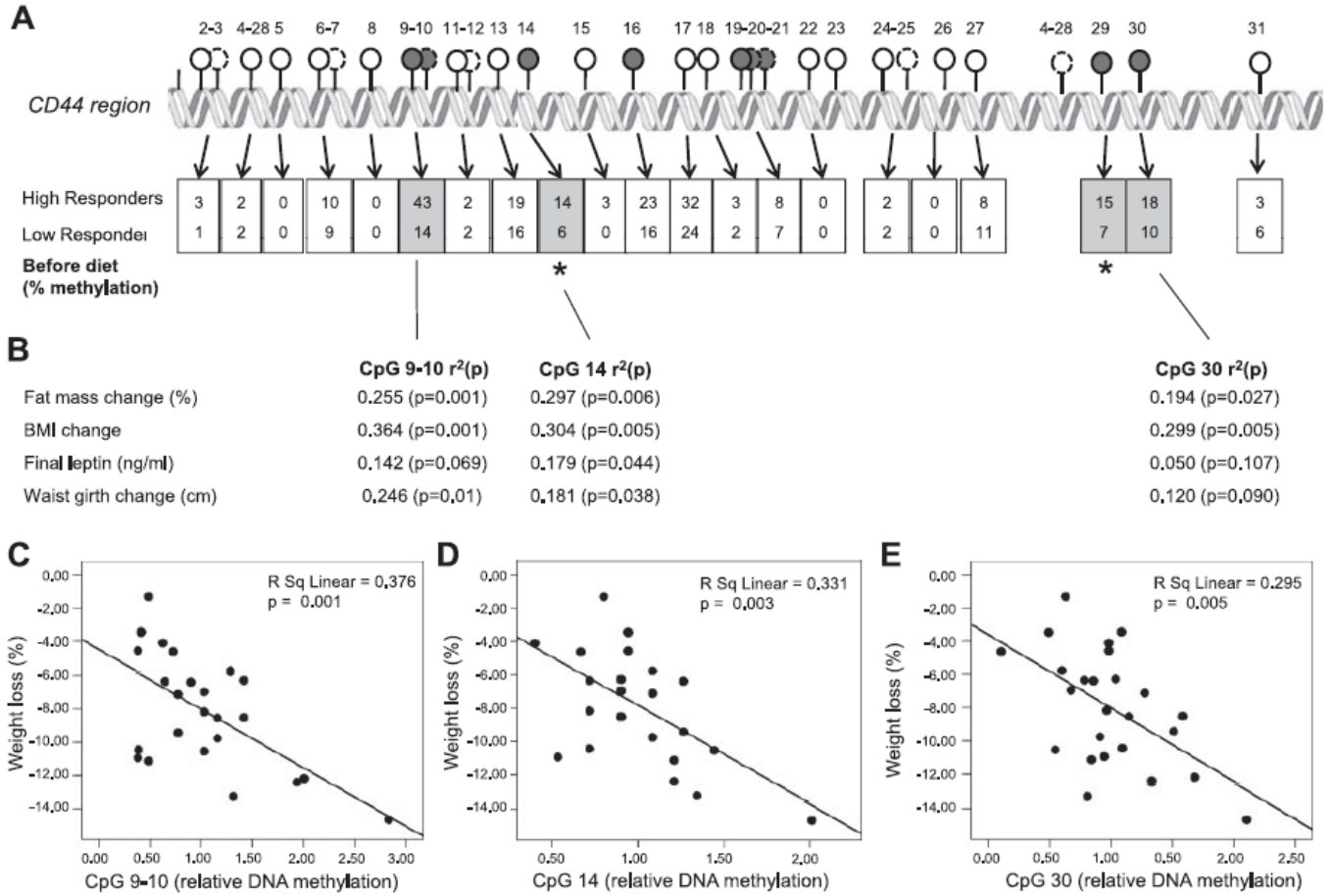


Figure 2. *A*) Baseline DNA methylation status (%) of 31 CpG sites of *CD44* in high and low responders to the hypocaloric diet by EpiTyper approach. Circle on a pole indicates that the site was quantified. Solid circles and shaded boxes indicate CpGs whose methylation levels correlated with other measurements. $*P < 0.05$; Mann-Whitney *U* test. *B*) Probability of association between baseline methylation levels of the indicated CpGs and the intervention-induced changes in different anthropometric and metabolic measurements (Pearson's correlation test). *C*) Correlation analysis between baseline methylation levels of one CpG located in *CD44* (CpG9–10) and weight loss during the intervention period (Pearson's correlation test). *D*) Correlation analysis between baseline methylation levels of CpG14 and weight loss during the intervention period (Pearson's correlation test). *E*) Correlation analysis between baseline methylation levels of CpG30 and weight loss during the intervention period (Pearson's correlation test).

CD44 is a cell-surface glycoprotein that acts as a receptor for hyaluronic acid. It is commonly expressed on hepatic Kupffer cells and infiltrating lymphocytes in liver and adipose tissue, and is considered an indirect marker of inflammation and early fibrosis

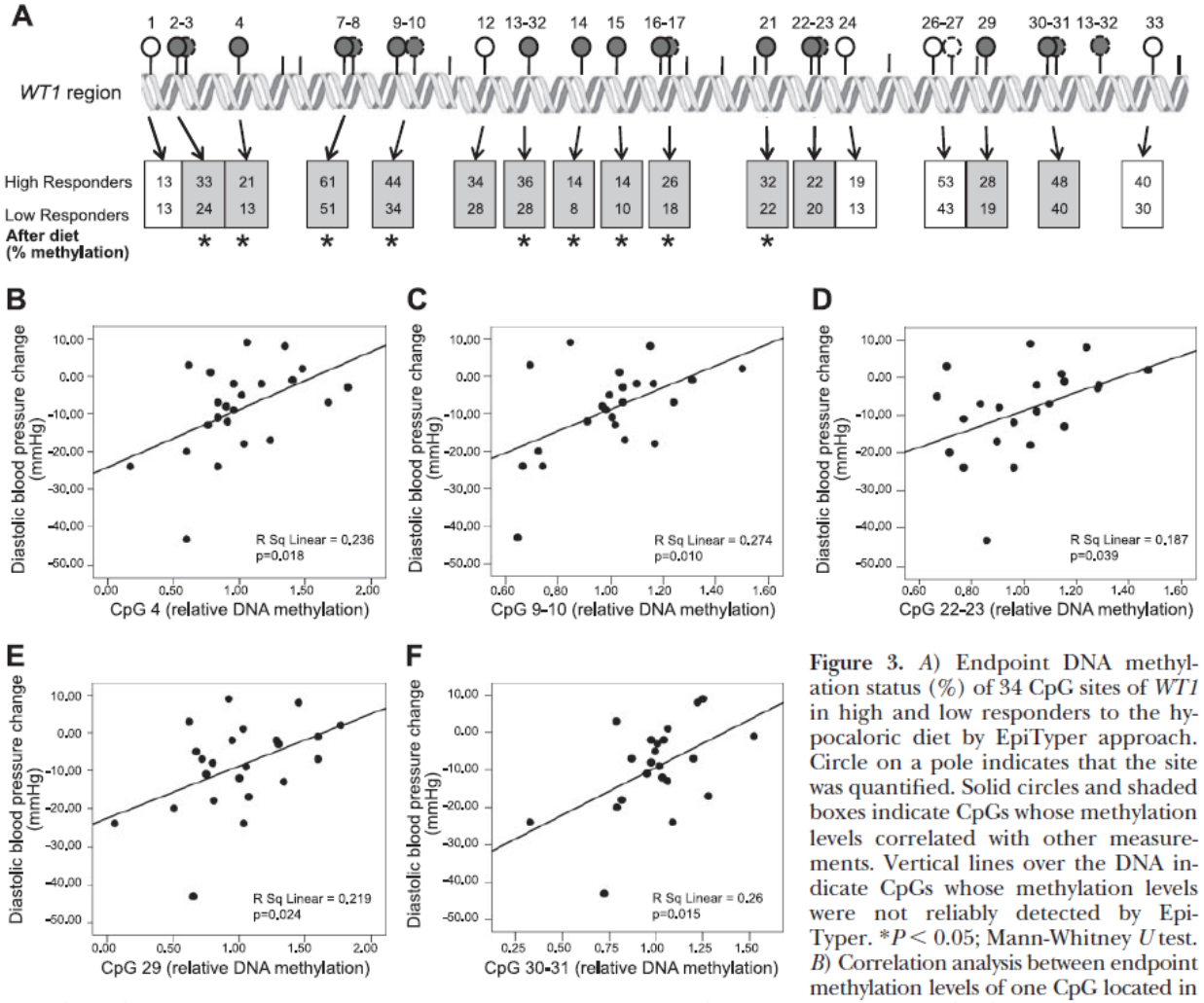


Figure 3. *A*) Endpoint DNA methylation status (%) of 34 CpG sites of *WT1* in high and low responders to the hypocaloric diet by EpiTyper approach. Circle on a pole indicates that the site was quantified. Solid circles and shaded boxes indicate CpGs whose methylation levels correlated with other measurements. Vertical lines over the DNA indicate CpGs whose methylation levels were not reliably detected by EpiTyper. $*P < 0.05$; Mann-Whitney *U* test. *B*) Correlation analysis between endpoint methylation levels of one CpG located in *WT1* (CpG4) and changes in the DBP during the intervention period (Pearson's correlation test). *C*) Correlation analysis between endpoint methylation levels of CpG9–10 and changes in DBP during the intervention period (Pearson's correlation test). *D*) Correlation analysis between endpoint methylation levels of CpG22–23 and the changes in the DBP during the intervention period (Pearson's correlation test). *E*) Correlation analysis between endpoint methylation levels of CpG29 and the changes in the DBP during the intervention period (Pearson's correlation test). *F*) Correlation analysis between endpoint methylation levels of CpG30–31 and the changes in the DBP during the intervention period (Pearson's correlation test).

An interesting gene that maintained the differences after the treatment was WT1. This gene encodes a Kruppellike zinc-finger protein that behaves as a transcription factor that can act as a tumor suppressor or an oncogene depending on the cell type in which it is expressed

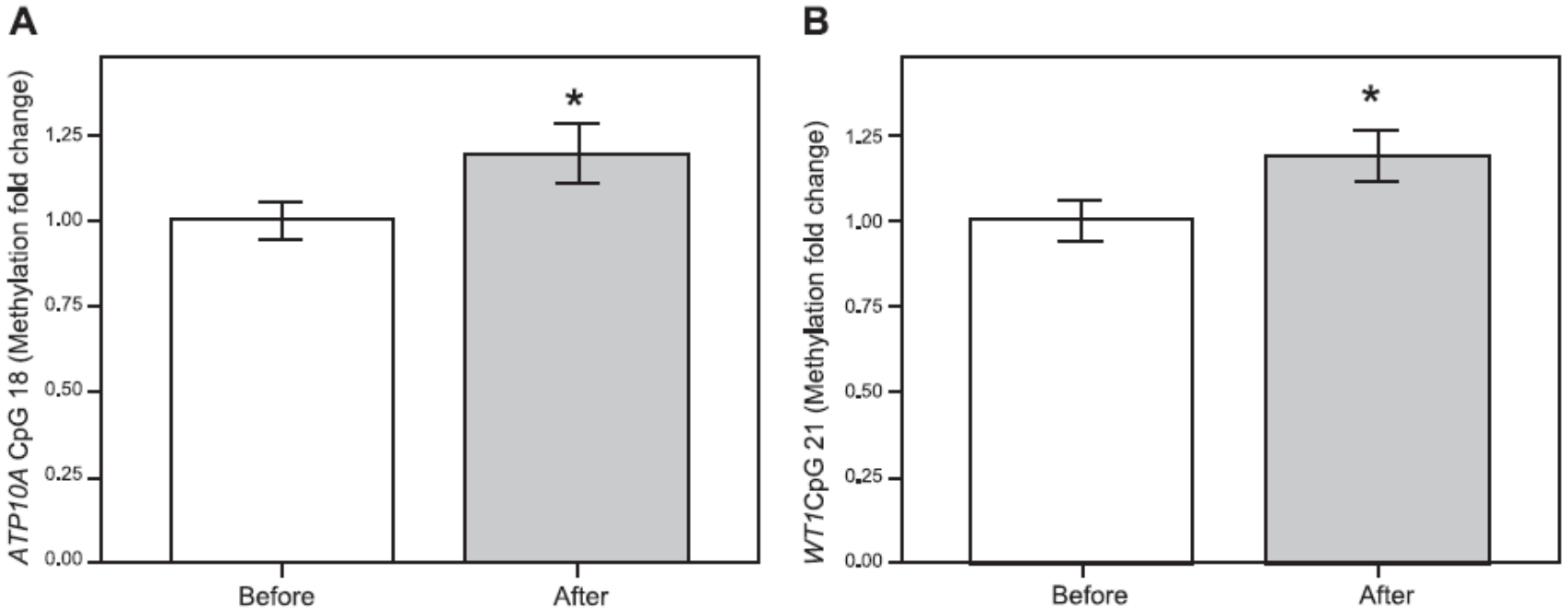


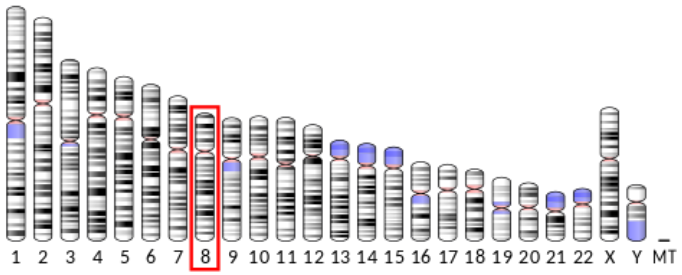
Figure 4. Variation of the methylation patterns of 2 CpG sites located in *ATP10A* (CpG18; *A*) and *WT1* (CpG 21; *B*) as a result of the nutrition intervention (comparing before *vs.* after treatment) by EpiTyper approach. $*P < 0.05$; paired *t* test.

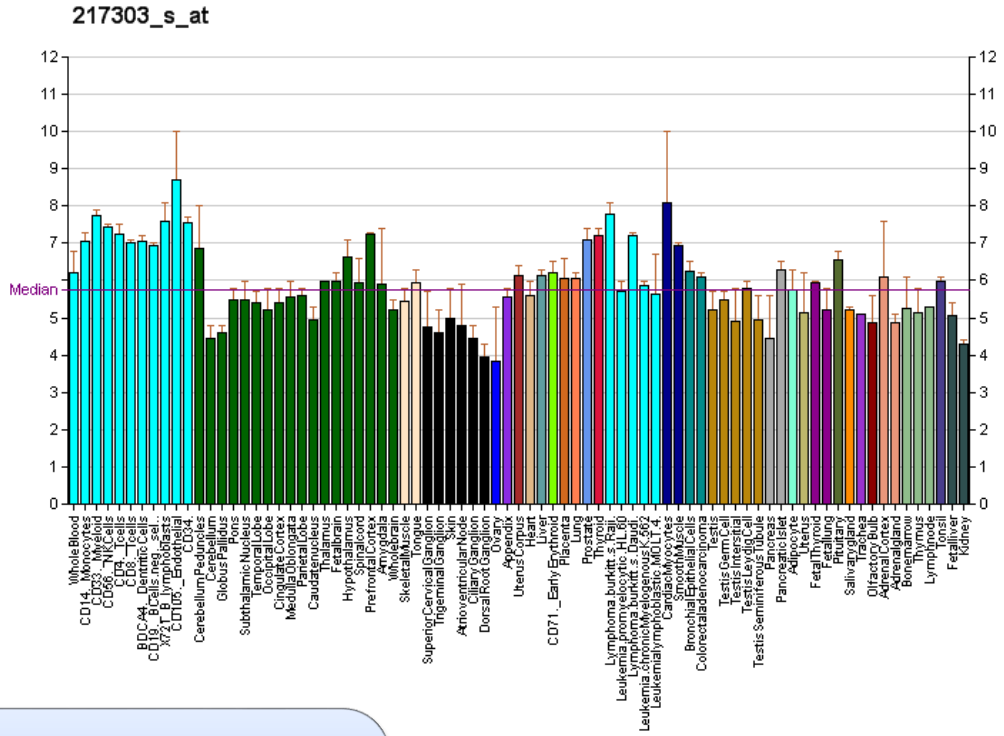
Concerning the last objective of this work, and taking into account only the high responders in the microarray assay, 5.8% of the CpGs (1570) were modified by the dietary treatment, which was much more than the CpGs modified in the low-responder group (194;0.7%).

This outcome clearly points out that the highresponder group is apparently more sensitive to diet induced epigenetic modifications, probably as a result of greater DNA methylation plasticity, which is defined as the methylation/demethylation changes that occur during growth and development

Effect of a diet containing folate and hazelnut oil capsule on the methylation level of the *ADRB3* gene, lipid profile and oxidative stress in overweight or obese women

- **Beta-3 adrenergic receptor**





It is located mainly in adipose tissue and is involved in the regulation of lipolysis and thermogenesis. Some β3 agonists have demonstrated antistress effects in animal studies, suggesting it also has a role in the central nervous system (CNS). β3 receptors are found in the gallbladder, urinary bladder, and in brown adipose tissue. Their role in gallbladder physiology is unknown, but they are thought to play a role in lipolysis and thermogenesis in brown fat.

Lima *et al. Clinical Epigenetics* (2017) 9:110

Aim of the study-Protocol

To evaluate the effect of a diet containing folate and hazelnut oil capsule on the methylation levels of the ADRB3 gene, lipid profile and oxidative stress in overweight and obese women.

This is a double-blind, placebo-controlled, intervention study

Adult women aged 20 to 59 years, who were overweight or obese. Each group consisted of 10 women, who received daily

vegetables and legumes containing folate for a total

period of 8 weeks.

The women were instructed to maintain the same weight, eating habits, and levels of physical activity that were found during the baseline evaluation.

G1

300 g of vegetables and legumes containing on average 191 µg/day of folate and 1 hazelnut oil capsule (25 g)

G2

300 g of vegetables and legumes containing, on average, 191 µg/day of folate and 1 placebo capsule

G3

300 g of vegetables and legumes containing on average 90 µg/day of folate and 1 hazelnut oil capsule (25 g)

G4

subjects were only followed-up and maintained their regular dietary habits

The hazelnut oil capsule that was offered was composed basically of monounsaturated fat (68%), rich in oleic acid.

Table 1 Primers used to analyze the methylation status

Gene	Primers		Annealing temperature
ADRB3	F	5′CCTTCCTTCTTTCCCTACCG3′	64 °C
	R	5′TGGTCTGGAGTCTCGGAGTC3′	

F forward primer, *R* reverse primer

Table 2 Anthropometric characteristics, methylation level, lipid profile and oxidative stress of women before and after intervention

Parameter	Before intervention		After intervention		
	Mean	SD	Mean	SD	*p* value
Weight (kg)	77.7	14.1	74.4	14	0.4013
Height (m)	1.59	1.1	1.59	1.1	–
BMI (kg/m^2)	30.5	5.3	29.2	5.1	0.3792
WC (cm)	0.94	0.12	0.90	0.12	0.3015
HC (cm)	1.14	0.12	1.01	0.11	0.2288
Waist-to-height ratio (WHtR; cm/m)	0.59	0.8	0.57	0.7	0.3471
Methylation level (%)	42.2	18.1	29.1	14.1	0.0006*
Total cholesterol (mg/dl)	201.6	46.2	194.8	48.1	0.5818
HDL-C (mg/dl)	44.3	9.6	50.7	9.2	0.0118*
LDL-C (mg/dl)	122.7	43.7	119.9	40.1	0.8103
Triglycerides (mg/dl)	146.7	80.6	150.5	67.7	0.8462
MDA	3.2	0.9	4	0.8	0.0029*
TAC (%)	41	13	53	12	0.0005*

malondialdehyde (MDA) as a lipid peroxidation marker], total antioxidant capacity (TAC)

Table 3 Methylation levels of the *ADRB3* gene, anthropometric data, and regular food intake of women of the post-intervention groups

Variables	Group 1 Mean ± SD	Group 2 Mean ± SD	Group 3 Mean ± SD	Group 4 Mean ± SD	*p* value
Methylation levels (%)	25.3 ± 5.2	34.7 ± 3.5	18.1 ± 2.6	38.3 ± 3.5	0.0030*
Weight	70.15 ± 2.93	80.56 ± 6;60	73.48 ± 2.08	75.91 ± 2.62	0.3207
Calories	1444.1 ± 114.5	1512.8 ± 67.7	1454.8 ± 100.1	1637.2 ± 100.6	0.4863
Folate	311.2 ± 7.1	335.4 ± 9.5	170.1 ± 14.0	96.4 ± 2.05	0.000*
Total fat (g)	48.9 ± 3.57	42.9 ± 2.61	49.57 ± 4.36	43.64 ± 4.1	0.4667
Total fat (%)	31.06 ± 1.84	25.5 ± 1.17	31.4 ± 2.92	24.2 ± 1.89	0.0037*
Saturated fat (g)	21.48 ± 2.22	21.32 ± 2.0	26.6 ± 1.91	19.24 ± 1.7	0.0748
Saturated fat (%)	13.8 ± 1.56	12.6 ± 1.08	16.9 ± 1.41	11.3 ± 1.58	0.0551
Monounsaturated fat (g)	33.6 ± 1.46	14.2 ± 0.41	36.02 ± 1.61	26.41 ± 2.98	0.0000*
Monounsaturated fat (%)	21.6 ± 1.26	15.3 ± 2.20	23.1 ± 1.51	8.6 ± 0.45	0.0000*
Polyunsaturated fat (g)	12.81 ± 1.7	6.76 ± 0.58	19.51 ± 1.66	9.21 ± 0.95	0.0000*
Polyunsaturated fat (%)	8.14 ± 0.96	5.3 ± 0.74	12.4 ± 1.34	4.05 ± 0.31	0.0000*
Trans fat (g)	0.58 ± 0.1	0.54 ± 0.08	0.55 ± 0.08	0.53 ± 0.11	0.9859

Table 6 Analysis of methylation levels, waist, WHtR, lipid profile, and oxidative stress, by group before and after intervention

Variables	Group 1		Group 2		Group 3		Group 4	
	Before	After	Before	After	Before	After	Before	After
Methylation levels (%)	41	25	55[a]	34[a]	35[b]	18[b]	42	38
Waist	0.95	0.92	0.97	0.95	0.9	0.84	0.96	0.96
WHtR	0.60	0.59	0.61	0.60	0.55	0.52	0.60	0.60
LDL-C	101.7	109.66	125.4	113.7	114.5	109.22	169	164
HDL-C	49.6	57.8	43	47.7	40.6	48.1	38.9	41.2
TAC (%)	35[c]	53[c]	41[d]	59[d]	46	49	40	46
MDA	3.45	4.2	3.14	3.87	3.03	3.76	3.23	3.45

Conclusions

In the present study, the dietary intervention with various quantities of folate from vegetables that were close to (but lower than) the DRI, as well as with adequate amounts of monounsaturated fatty acids from the hazelnut oil capsule, did not cause a statistically significant weight loss in the total sample of overweight and obese women

The dietary intervention followed the guidelines for weight maintenance. The results also showed a reduction in the methylation levels of the ADRB3 gene and an increase in the HDL-C values, as well as a decrease in the MDA and an increase in TAC, with both of the latter values being used to evaluate oxidative stress.

Combined Analysis of Stress- and ECM-Related Genes in Their Effect on Weight Regain

Nadia J.T. Roumans [1], Ping Wang [1,2], Roel G. Vink [1], Marleen A. van Baak [1], and Edwin C.M. Mariman [1]

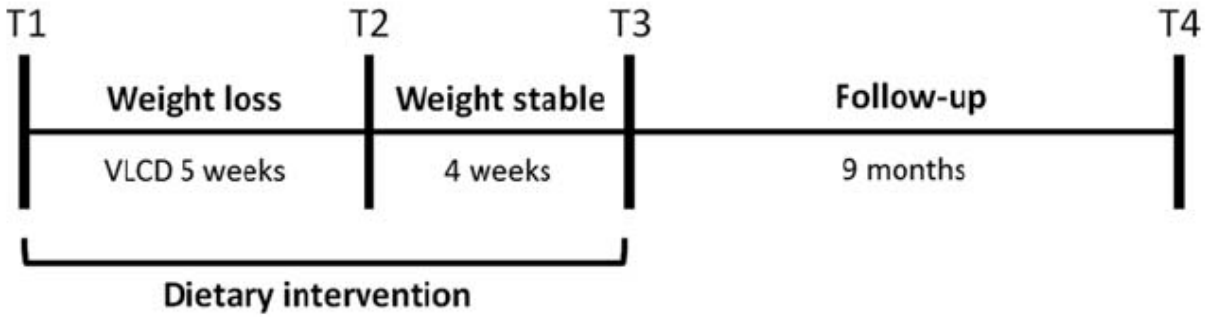


Figure 1 Schematic overview of the study design. Anthropometry, adipose tissue biopsies, and proteomics were performed at the start of the study (T1) and at the end of the weight-loss period (T2), weight-stable period (T3), and follow-up (T4). Transcriptomics was performed at T1, T2, and T3. The dietary intervention period is the weight-loss period and weight-stable period taken together.

Obesity (2017) **00**, 00–00. doi:10.1002/oby.22093

TABLE 1 Subject characteristics at study start, end of weight loss, end of weight-stable period, and end of follow-up

	Study start (T1)	End of WL (T2)	End of WS (T3)	End of follow-up (T4)
Sex (male/female)	12/14			
Age (y)	50.4 ± 1.5			
Weight (kg)	92.1 ± 1.9	83.1 ± 1.6[a]	82.9 ± 1.7	87.7 ± 2.0[a,b]
BMI (kg/m^2)	30.8 ± 0.4	27.8 ± 0.4[a]	27.7 ± 0.4	29.1 ± 0.5[a,b]
Hip circumference (cm)	111.0 ± 1.1	105.0 ± 1.0[a]	104.8 ± 1.0	105.4 ± 1.4[b]
Waist circumference (cm)	101.3 ± 1.6	93.5 ± 1.4[a]	94.6 ± 1.4	97.6 ± 1.7[a,b]
Body fat (%)	39.5 ± 1.6	34.8 ± 2.0[a]	33.7 ± 2.0[c]	36.0 ± 1.9[a,b]
Body fat (kg)	35.7 ± 1.2	28.4 ± 1.5[a]	27.3 ± 1.4[c]	31.0 ± 1.6[a,b]
Fat-free mass (kg)	55.7 ± 2.4	54.2 ± 2.3[a]	54.9 ± 2.4[c]	55.9 ± 2.4[d]

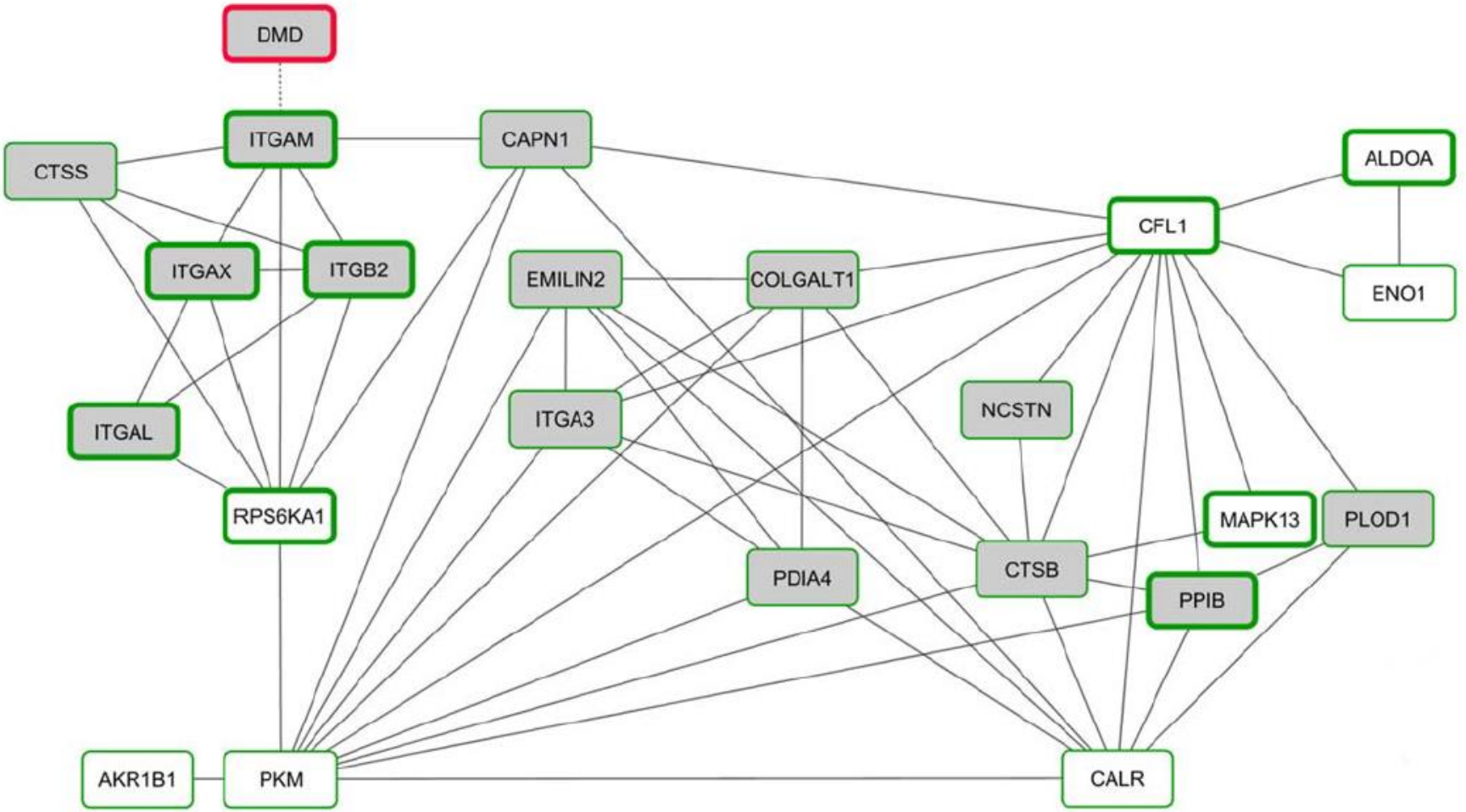


Figure 2 Correlation network between stress-related and ECM-related genes that strongly correlated with weight regain percentage ($P \leq 0.05$) during the weight stabilization phase. Correlations were made by using the Pearson correlation coefficient. Only genes that strongly correlated with each other ($|r| \geq 0.800$; $P \leq 0.001$) and with the weight regain percentage ($P \leq 0.05$) are depicted in this figure. White boxes, stress-related genes; grey boxes, ECM-related genes; solid line, positive correlation with other gene; dashed line, negative correlation with other gene; green outline, positive correlation with the weight regain percentage; red outline, negative correlation with the weight regain percentage; thick outline, strong correlation with weight regain percentage ($P \leq 0.01$). [Color figure can be viewed at wileyonlinelibrary.com]

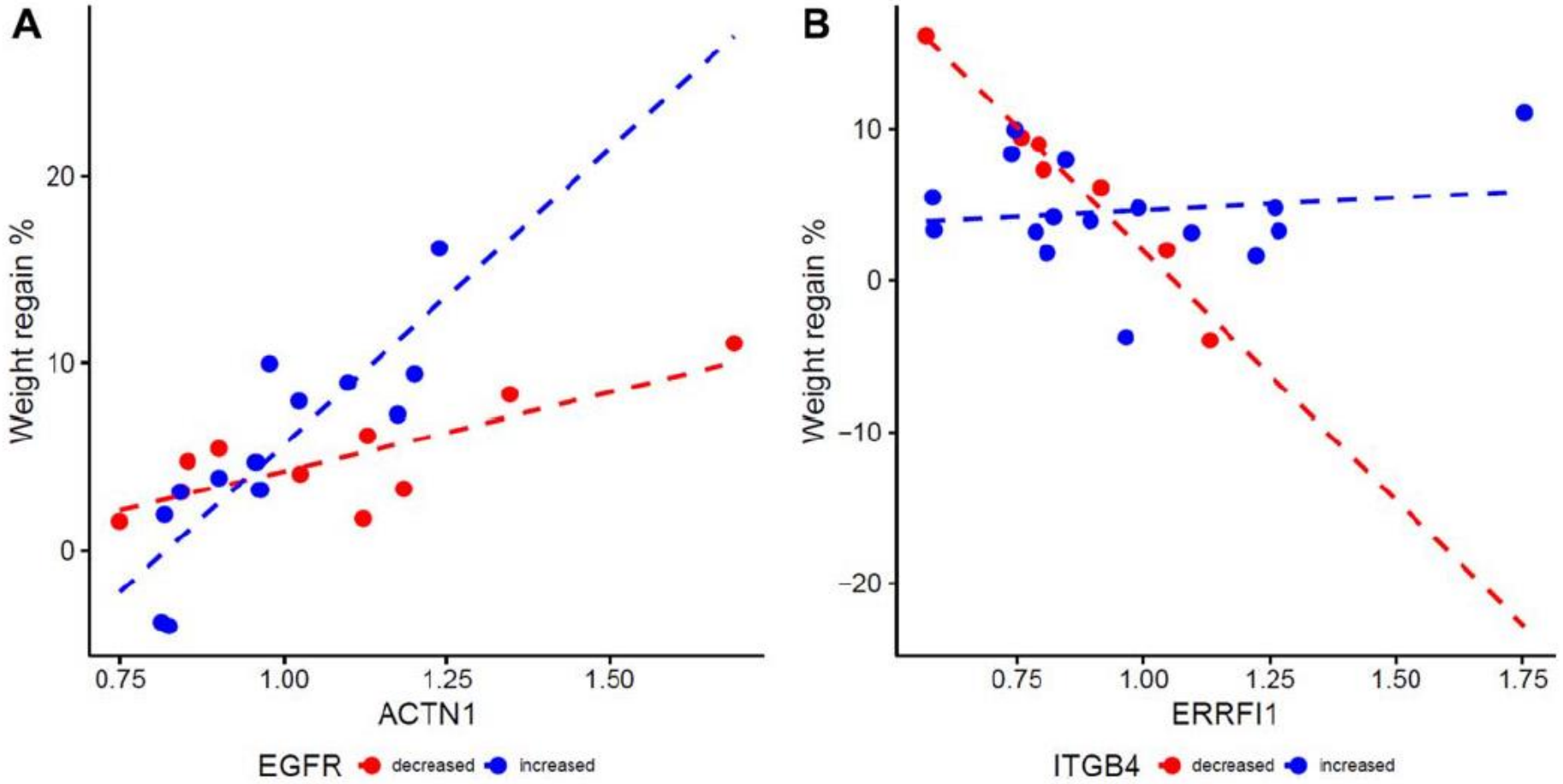


Figure 3 Correlation plot between the WR% and interaction of (**A**) the ECM-related gene *ACTN1* with the stress-related gene *EGFR* during WS and (**B**) the ECM-related gene *ITGB4* with the stress-related gene *ERRFI1* during WS. The lines represent the correlations between changes in WR% and gene expression of *ACTN1* or *ERRFI1* at decreasing (red line) or increasing (blue line) gene expression changes of *EGFR* or *ITGB4*.

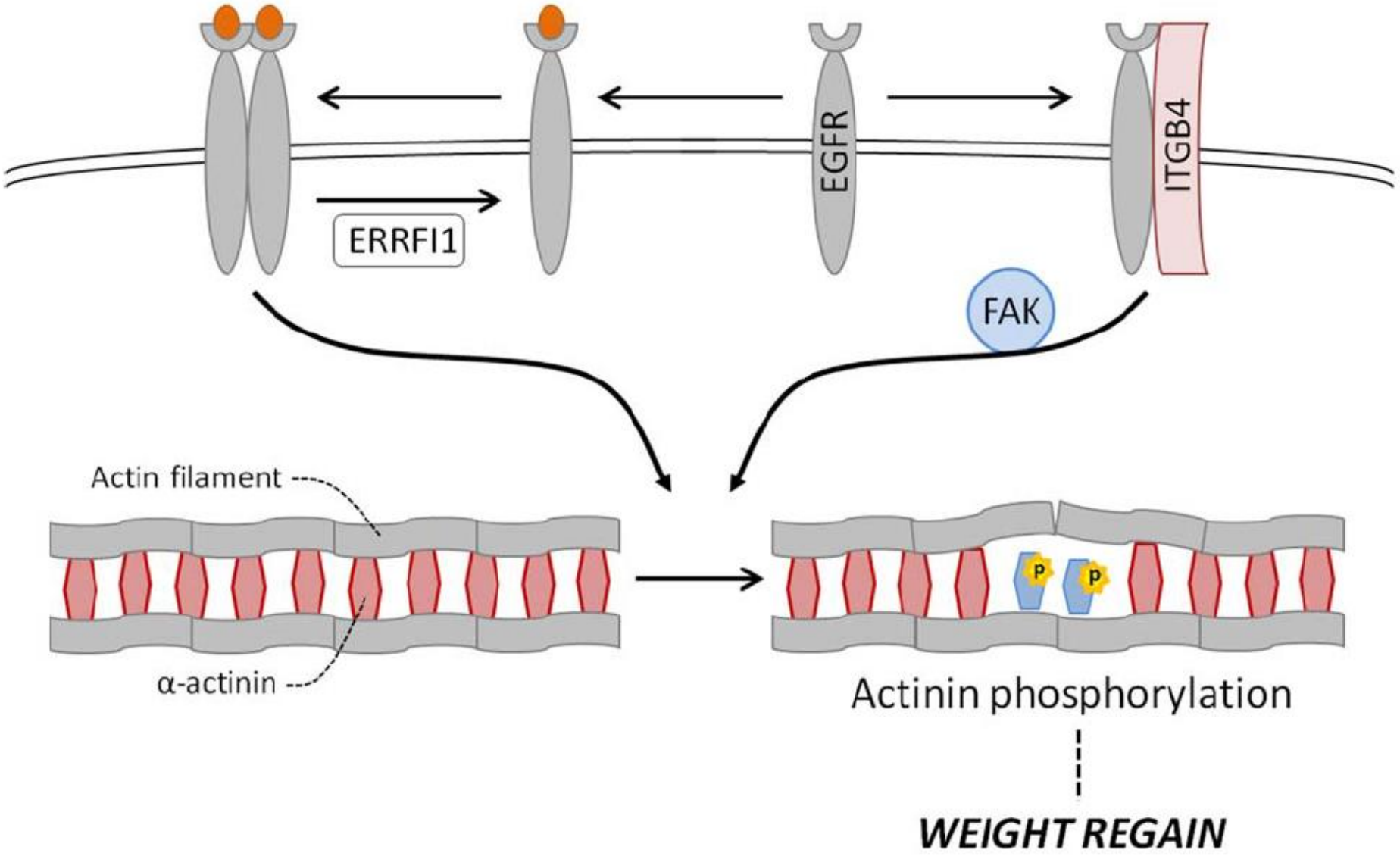


Figure 4 Schematic overview showing a possible role for α-actinin phosphorylation in the risk for weight regain via the epidermal growth factor signaling pathway based on the gene interactions correlating with WR%. [Color figure can be viewed at wileyonlinelibrary.com]

Conclusion

A coexpression network of stress-related genes and ECM-related genes correlating with the WR% could be constructed during the WS phase. The biological processes linked to this network were mainly focused on leukocyte-activity, ECM remodeling, actin cytoskeleton organization, and glucose handling. Interaction analysis between stress- and ECM-related genes revealed several gene combinations that were highly related to the WR%. In particular, certain interactions on gene expression of *ATF4* and the epidermal growth factor signaling pathway may have an influence on the risk for weight regain.