

REVIEW

Exercise training and DNA methylation in humans

S. Voisin,¹ N. Eynon,^{1,2} X. Yan^{1,2} and D. J. Bishop¹¹ Institute of Sport, Exercise and Active Living (ISEAL), Victoria University, Melbourne, Vic., Australia² Murdoch Childrens Research Institute, Royal Children's Hospital, Melbourne, Vic., Australia

Received 26 April 2014,
revision requested 14 June 2014,
revision received 9 September
2014,
accepted 18 October 2014
Correspondence: N. Eynon,
Institute of Sport, Exercise and
Active Living (ISEAL), Victoria
University, Melbourne, Vic. 8001,
Australia.
E-mail: nir.eynon@vu.edu.au

Abstract

The response to exercise training (trainability) has been shown to have a strong heritable component. There is growing evidence suggesting that traits such as trainability do not only depend on the genetic code, but also on epigenetic signals. Epigenetic signals play an important role in the modulation of gene expression, through mechanisms such as DNA methylation and histone modifications. There is an emerging evidence to show that physical activity influences DNA methylation in humans. The present review aims to summarize current knowledge on the link between DNA methylation and physical activity in humans. We have critically reviewed the literature and only papers focused on physical activity and its influence on DNA methylation status were included; a total of 25 papers were selected. We concluded that both acute and chronic exercises significantly impact DNA methylation, in a highly tissue- and gene-specific manner. This review also provides insights into the molecular mechanisms of exercise-induced DNA methylation changes, and recommendations for future research.

Keywords DNA methylation, epigenetics, exercise, sport, training.

The response to exercise training (i.e. trainability) has been shown to have a strong heritable component (Bouchard 2011, Eynon *et al.* 2011). In the health, risk, training And genetic (HERITAGE) family study, 742 healthy, sedentary participants, coming from families of white or black ancestry, underwent 20 weeks of standardized exercise training and the heritability of several exercise-related traits was measured (Bouchard *et al.* 1995). After adjustment for age, sex, body mass index (BMI) and baseline parameters, the heritability of the training responses for maximal oxygen uptake, submaximal exercise heart rate and submaximal exercise capacity were estimated as 47% (Bouchard *et al.* 1999), 34% (An *et al.* 2003) and 26% (Pérusse *et al.* 2001) respectively. Candidate gene association studies and genomewide association studies (GWAS) have subsequently led to the consensus that the heritable part of trainability can be attributed to the cumulative effect of many genetic

polymorphisms that have small individual effects (e.g. 0.5–7% each for trainability; Bouchard *et al.* 2011).

The most widely studied candidate genes for trainability are the angiotensin-converting enzyme (ACE) insertion/deletion of 287 base pair and the α -actinin-3 (ACTN3) R577X stop-codon polymorphisms. Several reports have shown that these polymorphisms are associated with both athletic performance outcomes and the training response (Eynon *et al.* 2009, 2012, 2013, Berman & North 2010, Puthuchearry *et al.* 2011). Recent GWAS findings, however, have created increased uncertainty regarding the role of these and other candidate genes in determining athletic performance and skeletal muscle traits (Garatachea *et al.* 2011, Broos *et al.* 2012, Folland *et al.* 2012, Vincent *et al.* 2012). Moreover, the few GWAS performed to date still require replication in other independent cohorts to ensure that the observed associations are not simply fortuitous. Thus, the uncritical view of

‘established’ genotypes affecting trainability is being increasingly challenged (Ehlert *et al.* 2013).

There is growing evidence suggesting that traits such as trainability not only depend on the genetic code, but also on epigenetics – that is transcriptional and post-transcriptional changes in gene expression that are not due to changes in the DNA sequence (Ehlert *et al.* 2013). Such processes are responsible for the establishment, maintenance and reversal of meta-stable transcriptional states (Bonasio *et al.* 2010). Indeed, epigenetic marks can be modified by the environment (e.g. exposure to toxic chemicals, nutrition) and differ significantly between different tissues, making them tissue specific (Zhang *et al.* 2013). There is also a growing evidence to support the hypothesis that some epigenetic modifications can be transgenerationally inherited (Bollati & Baccarelli 2010). Indeed, although much of the DNA methylation is cleared and re-established each generation (Reik *et al.* 2001), some DNA methylation marks escape this reprogramming and are transmitted epigenetically for at least three generations (Gluckman *et al.* 2007, Jirtle & Skinner 2007).

One major example of epigenetic modifications is the methylation of cytosine, usually at CpG dinucleotides, named DNA methylation. Regions rich in CpGs are called ‘CpG islands’ and are often found in the promoter region of active genes. When the CpGs of the promoter region are methylated, gene expression is silenced (Hashimshony *et al.* 2003). DNA methylation patterns are decisive in important processes such as development, X-chromosome inactivation and suppression of transposable elements and carcinogenesis (Bird 2002). DNA methylation status can be determined by various laboratory techniques, such as sodium bisulphite sequencing, methyl-sensitive polymerase chain reaction, chromatin immunoprecipitation or the use of DNA methylation array microchips (Shen & Waterland 2007). Three recent reviews and one current opinion have suggested that exercise training may induce changes in the methylation status of key genes involved in muscle function, thus possibly shaping a long-lasting favourable expression pattern for increased trainability (Sanchis-Gomar *et al.* 2012, Denham *et al.* 2013, Ehlert *et al.* 2013, Pareja-Galeano *et al.* 2014). These reviews are important and enhanced knowledge in the field. However, research into exercise and DNA methylation is rapidly developing, and it is important to update these reviews based on the latest research.

The present review aims to summarize current knowledge on the link between physical activity and DNA methylation in humans, to provide insights into the molecular mechanisms of exercise-induced DNA methylation and to suggest directions for future

research. The PubMed database was used to identify all relevant literature up until July 2014. Search terms were systematically a combination of ‘methylation’ with any of the following terms: ‘exercise’, ‘physical activity’, ‘sport’ and ‘exercise training’. Only papers focused on methylation of the DNA were included, and a total of 25 papers were selected. Titles and abstracts were systematically reviewed against the original inclusion. The main outcomes of these papers are summarized in Table 1.

Observational studies: a weak correlation between physical activity and DNA methylation

Because of the cost and the time of monitoring a cohort’s physical activity over a long period of time, many studies have chosen an observational design to determine the effect of chronic physical activity on DNA methylation status (Coyle *et al.* 2007, Slattery *et al.* 2007, Yuasa *et al.* 2009, Zhang *et al.* 2011a,b, 2012, Gomes *et al.* 2012, Lott *et al.* 2012, Morabia *et al.* 2012, Pirola *et al.* 2012, Ren *et al.* 2012, Luttrupp *et al.* 2013, White *et al.* 2013, Clarke-Harris *et al.* 2014). Ten of these observational studies focused on healthy populations (Coyle *et al.* 2007, Zhang *et al.* 2011a,b, 2012, Gomes *et al.* 2012, Morabia *et al.* 2012, Ren *et al.* 2012, Luttrupp *et al.* 2013, White *et al.* 2013, Clarke-Harris *et al.* 2014), while four studies chose diseased cohorts (Slattery *et al.* 2007, Yuasa *et al.* 2009, Lott *et al.* 2012, Pirola *et al.* 2012). Another two studies were genetic associations which, instead of directly looking at DNA methylation, focused on DNA methylation capacity and the chance of being an athlete (Terruzzi *et al.* 2011, Zarebska *et al.* 2013).

Physical activity and DNA methylation in diseased populations

Physical activity and the methylation status of cancer-related CpG sites in candidate genes. Aberrant DNA methylation patterns have been extensively described as triggers for carcinogenesis (You & Jones 2012). Cancer is commonly associated with increased methylation (termed hypermethylation) of tumour-suppressor genes (Ehrlich 2002). Indeed, 5–10% of normally unmethylated CpG promoter islands become abnormally methylated in various cancer genomes (Baylin & Jones 2011). In particular, tumour-suppressor genes (e.g. *p15*) as well as mismatch-repair genes (e.g. *hMLH1*) have been extensively shown to be *hypermethylated* in cancers (Mitchell *et al.* 2002, Yu *et al.* 2008). Long-term physical activity has been shown to reduce risk and mortality in various types of cancers,

particularly of the breast and colon (Ballard-Barbash *et al.* 2012). It is therefore interesting to explore whether some of the beneficial effects of exercise may be mediated by DNA methylation. Two studies have investigated the possible association between physical activity and the methylation status, measured by methylation-specific PCR, of several cancer-related genes in tumours from 106 gastric (Yuasa *et al.* 2009) and 1154 (Slattery *et al.* 2007) patients with colon cancer. No significant association was found between physical activity and the methylation levels of several genes (i.e. *MINT1*, *MINT2*, *MINT31*, *p16*, *CDX2*, *BMP2*, *ER* and *GATA5*; Slattery *et al.* 2007, Yuasa *et al.* 2009). However, the study by Yuasa *et al.* showed that methylation of *CACNA2D3* gene [a calcium channel gene often methylated in gastric cancer (Wanajo *et al.* 2008)] was higher in gastric carcinoma patients with no physical activity (45.5% of patients) than in those with at least 1 h of exercise per week before cancer onset (23.7% of patients; Yuasa *et al.* 2009).

Physical activity and the methylation of mitochondrial genes in disease conditions. Recently, the mitochondrial genome has been shown to undergo methylation in CpG dinucleotides, which in turn regulate mitochondrial function and gene transcription (Shock *et al.* 2011). One study investigated the correlations between the level of physical activity and the methylation status of the mitochondrial D-loop region in liver, as well as the mitochondrially encoded genes, NADH dehydrogenase 6 (*MT-ND6*) and cytochrome C oxidase I (*MT-CO1*), using methylation-specific PCR. These correlations were tested in a cohort comprising 45 non-alcoholic fatty liver patients and 18 controls, but were also tested in patients only. No correlation was found between physical activity and methylation of the D-loop region or *MT-CO1*. However, *MT-ND6* methylation was significantly moderately inversely correlated with the level of regular physical activity ($R = -0.30$; Pirola *et al.* 2012). This correlation was still significant, and even stronger, when the analysis was restricted to the patients ($R = -0.54$, $P < 0.05$). This study was the only one examining a possible exercise-induced methylation in the mitochondrial genome. It is therefore difficult to draw conclusions on this topic.

Physical activity and DNA methylation in healthy populations

Physical activity and the methylation status of cancer-related CpG sites. Apart from gene-specific hypermethylation, a genomewide reduction in DNA methylation (termed hypomethylation) is observed in most malignant cells and is associated with increased

cancer risk (Dawson & Kouzarides 2012). Global DNA *hypomethylation* increases genome instability by activating repetitive sequences (e.g. long interspersed nuclear elements [LINEs]), which are normally highly methylated (Sharma *et al.* 2010). Seven cross-sectional studies have investigated the correlation between physical activity and blood DNA methylation in five different healthy cohorts (Zhang *et al.* 2011a,b, 2012, Gomes *et al.* 2012, Morabia *et al.* 2012, Luttrupp *et al.* 2013, White *et al.* 2013).

Only one of these studies found a significant association between physical activity and global DNA methylation measured by luminometric methylation assay, in leucocytes from 509 elders; compared to a group with higher physical activity, decreased physical activity was associated with increased global methylation, and this remained significant after adjusting for multiple cardiovascular risk factors (Luttrupp *et al.* 2013). In another study, 647 women with a family history of breast cancer were asked to recall their level of physical activity during three time periods (childhood, teenage and the past year) and were divided into quartiles of activity (White *et al.* 2013). None of the quartiles were significantly associated with global DNA methylation measured in peripheral blood by pyrosequencing, but women who reported physical activity levels above the median at all three time periods had 0.33% higher methylation compared with those below the median at all time periods. Even if the difference was moderate, it suggested that exercise is associated with higher DNA methylation, which might decrease cancer risk.

Two studies conducted on a cohort of 165 cancer-free adults that performed DNA methylation analysis using pyrosequencing in peripheral white blood cells have examined the levels of physical activity in relation to methylation of LINE-1 and *IL-6* (Morabia *et al.* 2012, Zhang *et al.* 2012). Another two studies, from the same research group, investigated a correlation between physical activity and whole blood LINE-1 methylation, but in elders, using either pyrosequencing or qPCR (Zhang *et al.* 2011a,b). Finally, a case-control study with 45 women used methylation-specific PCR in breast epithelial cells to examine gene-specific methylation of *APC* and *RASSF1A* in relation to levels of physical activity (Coyle *et al.* 2007). None of these studies found significance. For one cohort, it was argued that low levels of physical activity (18.1 min per day of moderate plus vigorous physical activity, on average) may have made it difficult to identify a significant impact of physical activity on DNA methylation (Zhang *et al.* 2011a,b). The participants of another cohort were clustered into 'car drivers' and 'public transport users', because using the public transportation was hypothesized to require more physical activity and

Table 1 Summary and main outcomes of the studies reviewed

Article	Study design	<i>n</i>	Type of participants	Sex	Physical activity assessment
Observational studies					
Diseased populations					
Slattery <i>et al.</i> (2007)	Cross-sectional	1154	Patients with colon cancer	Men and women	Questionnaire on the intensity level of physical activity (for the current year, the past 10 years, and the past 20 years)
Yuasa <i>et al.</i> (2009)	Cross-sectional	106	Patients with gastric cancer	Men and women	Questionnaire on the frequency of recreational and voluntary physical exercise for health promotion (before cancer onset)
Pirola <i>et al.</i> (2012)	Cross-sectional	64	Non-alcoholic fatty liver patients and controls	Men and women	Questionnaire about all forms of regular physical activity surveyed at baseline by the time of liver biopsy
Lott <i>et al.</i> (2012)	Cross-sectional	82	Patients with schizophrenia	Men and women	Questionnaire on the frequency and duration of strenuous, moderate and mild activities (average on the past week)
Healthy populations					
Gomes <i>et al.</i> (2012)	Cross-sectional	126	Physically independent elders	Men and women	Average of 1 week of steps per day measured by a pedometer
Morabia <i>et al.</i> (2012)	Case-control	165	Young cancer-free adults	Men and women	Questionnaire on the frequency and duration of 26 leisure-time, job-related and home physical activities determined by the National Human Activities Patterns Survey (average of the past year)
Zhang <i>et al.</i> (2011b)	Cross-sectional	131	Healthy elders	Men and women	Min per day spent in moderate and vigorous physical activity measured by an accelerometer during 4 days
Zhang <i>et al.</i> (2011a)	Cross-sectional	161	Healthy elders	Men and women	Average of 4 days of min per day spent in moderate and vigorous physical activity measured by an accelerometer
Zhang <i>et al.</i> (2012)	Cross-sectional	165	Cancer-free adults	Men and women	Questionnaire on the frequency and duration of 26 leisure-time, job-related and home physical activities determined by the National Human Activities Patterns Survey (average on the past year)
Luttrupp <i>et al.</i> (2013)	Cross-sectional	509	Elders	Men and women	Questionnaire about lighter and heavier (sweat-inducing) physical activity per week
Clarke-Harris <i>et al.</i> (2014)	Follow-up	40	Children	Boys and girls	Measured annually by accelerometer, between 5 and 14 years of age
Ren <i>et al.</i> (2012)	Case-control	500	Tai Chi practitioners and non-practitioners	Women	Criteria to be included in the Tai Chi cohort: practising Tai Chi ≥ 3 years ≥ 1 h week ⁻¹
Coyle <i>et al.</i> (2007)	Cross-sectional	45	Cancer-free pre and post menopausal women	Women	Questionnaire on intensity, frequency and duration of occupation, household and sports (for the past year, the past 5 years, and over a lifetime)
White <i>et al.</i> (2013)	Cross-sectional	647	Middle-aged with family history of breast cancer	Women	Questionnaire on intensity, frequency and duration of occupation, household and sports (for the past year, at ages 5–12 and 13–19)

Targeted genes	Tissue used for DNA methylation assay	DNA methylation method	Main results
<i>MINT1</i> , <i>MINT2</i> , <i>MINT31</i> , <i>p16</i> and <i>bMLH1</i> (CpG islands)	Colon tumours	Bisulphite treatment of DNA + methylation-specific PCR	No significant correlation between physical activity and number of methylated markers
<i>CDX2</i> , <i>BMP2</i> , <i>p16</i> , <i>CACNA2D3</i> , <i>ER</i> and <i>GATA5</i>	Gastric tumours	Bisulphite treatment of DNA + methylation-specific PCR	Significant correlation between physical activity and <i>CACNA2D3</i> methylation
<i>MT-ND6</i> , <i>MT-CO1</i> and <i>D-loop</i>	Liver	Bisulphite treatment of DNA + methylation-specific PCR	Significant inverse correlation between <i>MT-ND6</i> methylation and physical activity
<i>COMT</i>	Peripheral blood	Bisulphite treatment of DNA + pyrosequencing	(1) Positive correlation between physical activity and <i>COMT</i> promoter methylation for Met/Met homozygotes (2) Negative correlation for Val/Val homozygotes (3) No correlation for Met/Val heterozygotes
Global DNA methylation	Peripheral blood white blood cells	Dosage of methyl by Imprint Methylated DNA Quantification	No significant correlation between physical activity and global DNA methylation
<i>LINE-1</i> and <i>IL-6</i>	Peripheral blood white blood cells	Bisulphite treatment of DNA + pyrosequencing	No significant correlation between public transport users and car drivers regarding <i>LINE-1</i> or <i>IL-6</i> methylation
Global DNA methylation	Peripheral blood	Bisulphite treatment of DNA + qPCR	No significant correlation between physical activity level and <i>LINE-1</i> methylation
Global DNA methylation	Peripheral blood	Bisulphite treatment of DNA + pyrosequencing	No significant correlation between physical activity level and <i>LINE-1</i> methylation
<i>LINE-1</i> and <i>IL-6</i>	Peripheral blood white blood cells	Bisulphite treatment of DNA + pyrosequencing	No significant correlation between physical activity and <i>LINE-1</i> or <i>IL-6</i> methylation
Global DNA methylation	Leucocytes	Luminometric methylation assay	Significant negative association between exercise activity group and global methylation
<i>PGC-1α</i>	Whole blood	Bisulphite treatment of DNA + pyrosequencing	No interaction between physical activity and methylation at several loci in the promoter of <i>PGC-1α</i>
<i>RAD50</i> , <i>ESR1</i> , <i>WRN</i> , <i>ERCC1</i> , <i>G6PD</i> , <i>17p</i> and <i>Xp13</i>	Mouthwash	Bisulphite treatment of DNA + MALDI-TOF MS	Age-related DNA methylation changes in 6 CpG sites (<i>17p_7</i> , <i>Xp13_1</i> , <i>Rad50_2</i> , <i>Rad50_10</i> , <i>G6PD_6</i> , and <i>G6PD_7</i>) significantly slowed down by Tai Chi practice
<i>APC</i> and <i>RASSF1A</i>	Breast epithelial cells	Bisulphite treatment of DNA + methylation-specific PCR	No significant correlation between physical activity and <i>APC</i> or <i>RASSF1A</i> methylation
<i>LINE-1</i>	Peripheral blood	Bisulphite treatment of DNA + pyrosequencing	Women with physical activity above the median at all three time periods had significantly increased methylation compared with those below the median at all three time periods

Table 1 (continued)

Article	Study design	<i>n</i>	Type of participants	Sex	Physical activity assessment
Elite athletes					
Zarebska <i>et al.</i> (2013)	Case-control	2684	Athletes and controls	Men and women	No physical activity assessment
Terruzzi <i>et al.</i> (2011)	Case-control	131	Elite athletes and sedentary controls	?	No physical activity assessment
Interventional studies					
Acute exercise					
Barrès <i>et al.</i> (2012)	Acute exercise cohort	14	Healthy young sedentary adults	Men and women	VO _{2peak} test under fasting conditions
	Exercise intensity cohort	8		Men	Two isocaloric acute exercise trials at 40% VO _{2peak} (low-intensity) and 80% VO _{2peak} (high intensity; exercise intensity cohort)
Chronic exercise					
Candidate gene studies					
Alibegovic <i>et al.</i> (2010)	–	20	Young healthy Caucasian men	Men	9 days of bed rest + 4 weeks of retraining (30 min day ⁻¹ , 6 days week ⁻¹ , at 70% VO _{2max})
Nakajima <i>et al.</i> (2010)	RCT	383	Healthy elders	Men and women	6-month-long supervised high-intensity interval walking regimen
Bryan <i>et al.</i> (2012)	–	64	Healthy sedentary	Men and women	12 months of unsupervised intervention to increase physical activity: self-reports on physical activity at 3, 6, 9 and 12 months + VO _{2max} test at baseline and at 12 months
Kulkarni <i>et al.</i> (2012)	–	40	Type 2 diabetic patients and normal-glucose tolerant controls	Men and women	4-month-long unsupervised walking exercise (5 h week ⁻¹)

Targeted genes	Tissue used for DNA methylation assay	DNA methylation method	Main results
<i>MTHFR</i>	No DNA methylation assay		Genetic variants impairing the catalytic efficiency of <i>MTHFR</i> , significantly more frequent in sprint-strength and strength athletes compared with controls
<i>MTHFR</i> , <i>MTR</i> , <i>MTRR</i> , <i>BHMT</i> , and <i>CBS</i>	No DNA methylation assay		Genetic variants impairing the catalytic efficiency of <i>MTHFR</i> , <i>MTR</i> and <i>MTRR</i> significantly more frequent in elite athletes compared with controls
Global DNA methylation	Muscle	Luminometric methylation assay	Decrease in global DNA methylation after acute exercise due to gene-specific hypomethylation
<i>PGC-1α</i> , <i>TFAM</i> , <i>PDK4</i> , <i>CS</i> , <i>PPAR-δ</i> , <i>MEF2A</i> , <i>MYOD1</i> and <i>GAPDH</i>		MeDIP + methylcytosine capture: qPCR	(1) High-intensity but not low-intensity exercise causes hypomethylation (2) Exercise-induced hypomethylation requires Ca^{2+} from the endoplasmic reticulum (3) Hypomethylation does not require circulating factors
<i>PGC-1α</i>	Muscle	Bisulphite treatment of DNA + sequencing	(1) Significant increase of 1 CpG site in the promoter of <i>PGC-1α</i> after 9 days of bed rest (2) Methylation level correlated with a decrease in <i>PGC-1α</i> mRNA levels (3) Initial methylation level of <i>PGC-1α</i> not restored after retraining but <i>PGC-1α</i> expression level restored after retraining
<i>ASC</i> and <i>p15</i>	Peripheral blood	Bisulphite treatment of DNA + pyrosequencing	Significantly higher methylation of <i>ASC</i> in the exercised subjects compared with controls
Average methylation of 45 CpG sites across 27 genes related to breast cancer + average methylation genomewide	Saliva	Bisulphite treatment of DNA + Infinium HumanMethylation27 BeadChip microarray	(1) Baseline gene-specific methylation significantly negatively correlated with VO_{2max} , but not with self-reported physical activity (2) Genomewide and gene-specific increase in DNA methylation after the exercise intervention (3) No significant correlation between change in VO_{2max} and genomewide or gene-specific methylation after intervention (4) Significant negative correlation between self-reported physical activity and gene-specific methylation after intervention
<i>PDK4</i>	Muscle	Bisulphite treatment of DNA + sequencing	(1) Significant correlation between <i>PDK4</i> expression and exercise in controls only (2) Significantly higher methylation in the promoter of <i>PDK4</i> at baseline in Type 2 diabetic patients compared with controls

Table 1 (continued)

Article	Study design	<i>n</i>	Type of participants	Sex	Physical activity assessment
Global and genomewide studies					
Duggan <i>et al.</i> (2014)	RCT	298	Overweight sedentary	Women	12 months of supervised nutrition and exercise intervention, with 82 diet restriction, 70 moderate-to-vigorous intensity exercise and 87 diet & exercise
Rönn <i>et al.</i> (2013)	–	23	Healthy young sedentary adults	Men	6 month of supervised endurance exercise training (3 h week ⁻¹)
Nitert <i>et al.</i> (2012)	–	28	Healthy young sedentary adults	Men	6-month-long supervised endurance exercise training (3 h week ⁻¹)
Zeng <i>et al.</i> (2011)	RCT	12	Physically inactive postmenopausal women diagnosed with stage 0–IIIA breast cancer	Women	6-month-long supervised moderate-intensity aerobic exercise (150 min week ⁻¹)

APC, adenomatous polyposis coli; ASC, PYD and CARD domain containing; BDNF, brain-derived neurotrophic factor; BMP2, bone morphogenetic protein 2; BHMT, betaine: homocysteine methyltransferase; CACNA2D3, calcium channel, voltage-dependent, alpha 2/delta subunit 3; CBS, cystathionine β -synthase; CDX2, caudal type homeobox 2; COMT, catechol-O-methyl transferase; CS, citrate synthase; ER, oestrogen receptor; ERCC1, excision repair cross-complementing rodent repair deficiency, complementation group 1; ESRI, oestrogen receptor 1; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; GATA5, GATA binding protein 5; IL-6, interleukin-6; LINE-1, long interspersed nuclear element-1; MeDIP, methylated DNA immunoprecipitation; MEF2A, myocyte enhancer factor 2A; MT-ND6, mitochondrially encoded NADH dehydrogenase 6; MT-CO1, cytochrome C oxidase I; MTHFR, 5, 10-methylenetetrahydrofolate reductase; MTR, methionine synthase; MTRR, methionine synthase reductase; MYOD1, myogenic differentiation 1; PDK4, pyruvate dehydrogenase kinase, isozyme 4; PGC-1 α , peroxisome proliferator-activated receptor gamma coactivator 1-alpha; PPAR- δ , peroxisome proliferator-activated receptor δ ; p15, cyclin-dependent kinase 4 inhibitor B; RAD50, RAD50 homologue; RASSF1A, Ras association (RalGDS/AF-6) domain family member; RCT, randomized controlled trial; TFAM, mitochondrial transcription factor A; WRN, Werner syndrome, RecQ helicase-like.

energy expenditure than car use, but no significant difference was found between the two groups with respect to global DNA methylation (Morabia *et al.* 2012). However, this does not seem too surprising

considering that after adjustment for age, gender, race/ethnicity and BMI, ‘car drivers’ and ‘public transport users’ had similar levels of overall physical activity.

Targeted genes	Tissue used for DNA methylation assay	DNA methylation method	Main results
LINE-1	Peripheral blood	Bisulphite treatment of DNA + pyrosequencing	<ol style="list-style-type: none"> (1) No association between baseline characteristics of participants or previously measured biomarkers, and LINE-1 methylation (2) No difference in LINE-1 methylation between intervention group and control (3) Magnitude of weight-loss not associated with LINE-1 methylation at 12-months
Genomewide	Adipose tissue	Bisulphite treatment of DNA + Infinium HumanMethylation450 BeadChip microarray and pyrosequencing	<ol style="list-style-type: none"> (1) Global increase in DNA methylation after the exercise intervention (2) Differential mRNA expression in 1/3 of the gene regions with altered DNA methylation, including <i>RALBP1</i>, <i>HDAC4</i> and <i>NCOR2</i> (3) 18 obesity and 21 type 2 diabetes candidate genes had CpG sites with differences in adipose tissue DNA methylation in response to exercise
Genomewide	Muscle	MeDIP	<ol style="list-style-type: none"> (1) Significant correlation between the methylation of 134 genes and physical activity (86% of genes hypomethylated) (2) 4 biological pathways of genes that both alter methylation due to exercise and exhibited increased mean expression values after exercise (purine metabolism, insulin signalling, ErbB signalling, and progesterone-mediated oocyte maturation)
Genomewide	Blood	Bisulphite treatment of DNA + Infinium HumanMethylation27 BeadChip microarray	Significant correlation between the methylation of 43 genes and physical activity

Exercise and the methylation status of age-related CpG sites. To date, only one study has investigated the effect of regular physical activity on age-related methylation changes (Ren *et al.* 2012). In this study,

DNA methylation in saliva, measured by MALDI-TOF MS, of 60 CpG sites across seven loci known to be influenced by age was compared between a cohort of 237 Tai Chi (a mind–body exercise) practitioners

and a matched control cohort of 263 people who had never practised Tai Chi, but whose regular physical activity level was similar to the Tai Chi cohort. Those in the Tai Chi cohort showed a lower DNA methylation (mean methylation difference ranging from 0.019 to 0.106 across the six CpGs) at six of the investigated CpG sites (17p_7, Xp13_1, Rad50_2, Rad50_10, G6PD_6 and G6PD_7). However, there were some limitations to this study. First of all, 26% of the Tai Chi practitioners reported that they took up Tai Chi to help with specific health problems, and they indeed had poorer health compared to the controls. They had higher hypertension and anxiety, which have been shown to influence DNA methylation (Adeyemo *et al.* 2009, Smolarek *et al.* 2010). Furthermore, the history and frequency of Tai Chi practice varied greatly. Finally, the authors did not adjust their statistics for multiple comparisons, increasing the chance of false positives. Nevertheless, this study had a good sample size and suggests that specific types of exercise might affect the epigenome.

Exercise and the methylation status of PGC-1 α and COMT. One study measured annually the methylation of several sites in the promoter of *PGC-1 α* in 40 children between 5 and 14 years of age, to test whether methylation of *PGC-1 α* in blood could predict adiposity up to 14 years (Clarke-Harris *et al.* 2014). While four sites in the promoter of *PGC-1 α* could predict adiposity, no interaction between physical activity and *PGC-1 α* promoter methylation was found.

Another study has examined the impact of physical activity level on methylation of the catechol-O-methyl transferase (*COMT*) promoter region in blood, with respect to the genotype at the 158Val/Met *COMT* variant (Lott *et al.* 2012). A weak, positive, correlation between physical activity and *COMT* promoter methylation ($r^2 = 0.19$ at one CpG site and $r^2 = 0.21$ at a second CpG site, $n = 18$) was found for Met/Met homozygotes, whereas a moderate negative correlation ($r^2 = 0.27$ at one CpG site and $r^2 = 0.39$ at a second CpG site, $n = 28$) was found for Val/Val homozygotes, and heterozygotes showed no correlation at all.

Elite athlete status and DNA methylation capacity

A recent case–control study compared polymorphisms in five genes thought to be involved in the capacity of DNA methylation between 77 elite athletes and 54 non-athletes (Terruzzi *et al.* 2011). Heterozygotes for variants known to impair the catalytic efficiency of methylenetetrahydrofolate reductase (*MTHFR*), methionine synthase (*MTR*) and methionine synthase reductase (*MTRR*) [three enzymes involved in the metabolic

cycle providing methyl groups to the DNA (Ulrey *et al.* 2005)] were significantly more frequent in elite athlete compared to non-athletes. However, the participant's ethnic background was not reported; this is possibly an important omission, as this has been shown to affect genotype frequencies. A second case–control study in a larger sample of 1144 athletes and 1540 controls supported the previous findings on the *MTHFR* 1298A>C polymorphism (Zarebska *et al.* 2013). AC heterozygotes were over-represented in sprint–strength and sprint athletes compared with controls, suggesting that the C allele is beneficial to sprint/power performance. But it is still unclear whether and to what extent this effect is due to changes in DNA methylation.

Summary

Regardless of the health status of the studied population, observational studies consistently show a weak correlation between physical activity and global or gene-specific DNA methylation. However, in most of these studies, physical activity was assessed via questionnaires at the time the study was conducted (Slattery *et al.* 2007, Pirola *et al.* 2012, Luttrupp *et al.* 2013), before cancer onset (Yuasa *et al.* 2009), for the past week (Lott *et al.* 2012), for the past year (Coyle *et al.* 2007, Morabia *et al.* 2012, Zhang *et al.* 2012, White *et al.* 2013), the past 5 years (Coyle *et al.* 2007), the past 10 or 20 years (Slattery *et al.* 2007) and over a lifetime (Coyle *et al.* 2007). Therefore, the possibility of recall errors from the participants may have generated an important bias (Matthews 2002, Lissner *et al.* 2004). In a review of studies comparing direct and objective measures, with indirect and subjective measures of physical activity, correlations were generally low to moderate. In four studies, physical activity level was assessed by an accelerometer worn for five consecutive days (Zhang *et al.* 2011a,b, Clarke-Harris *et al.* 2014), or by a pedometer worn for 1 week (Gomes *et al.* 2012). Moreover, these studies have assessed the methylation status in selected tissues that is blood, liver, saliva, breast epithelium, gastric and colon tumours. Hence, it cannot be excluded that a correlation between DNA methylation and physical activity in other unstudied tissues, such as adipose tissue or muscle, exists.

Furthermore, these studies may have all suffered from a typical shortcoming of observational studies, namely selection bias (Jepsen *et al.* 2004, Hannan 2008). In an observational design, the absence of randomization of the participants leads to a possible large and unobserved difference in their characteristics. If one of these characteristics is linked to the measured outcome, the estimate of the impact of the

studied factor (here, physical activity level) on the outcome (here, DNA methylation level) may be biased. Common examples of these factors (referred to as confounders) are sex, age, BMI and smoking. For instance, inflammation is known to promote DNA hypermethylation, making it an important confounder (Ingrosso & Perna 2009). In addition, selection bias could have resulted in very homogeneous samples in which there was little variability in physical activity levels, thus reducing power to detect a significant association between physical activity and DNA methylation. A better approach to address the issue of the influence of exercise on DNA methylation is to set up an interventional design where the participants' physical activity is monitored.

Interventional studies

DNA methylation changes following an acute exercise training

Mitochondria generate the cell's supply of adenosine tri phosphate (ATP) and are therefore crucial for skeletal muscle contraction. Skeletal muscle mitochondrial function adapts to acute exercise and endurance training, and the volume of mitochondria increases (Tonkonogi & Sahlin 2002, Bishop *et al.* 2010, 2013, Little *et al.* 2011). Only one study, conducted in two

cohorts of 14 young sedentary healthy men and women and 8 men, respectively, has quantified the influence of an acute bout of high-intensity exercise on skeletal muscle DNA methylation changes in genes involved in mitochondrial function and fuel usage, as measured by qPCR following methylated DNA immunoprecipitation (MeDIP; Barrès *et al.* 2012). The investigated genes were peroxisome proliferator-activated receptor gamma coactivator 1-alpha (*PGC-1α*), pyruvate dehydrogenase kinase, isozyme 4 (*PDK4*), mitochondrial transcription factor A (*TFAM*), peroxisome proliferator-activated receptor δ (*PPAR-δ*), citrate synthase (*CS*), myocyte enhancer factor 2A (*MEF2A*) and myogenic differentiation 1 (*MYOD1*). *PGC-1α* is known to be a key regulator of mitochondrial biogenesis, fatty acid oxidation and insulin sensitivity in skeletal muscle and is also upregulated in response to exercise (Lira *et al.* 2010). *PDK4*, a key gene in skeletal muscle metabolism that is associated with hyperglycaemia (Jeoung & Harris 2008), is known to be increased in human skeletal muscle after both short-term high-intensity and prolonged low-intensity exercise (Pilegaard & Neufer 2004).

While the muscle-specific *MYOD1* showed no significant change in response to exercise, *MEF2A* and *CS* methylation levels decreased in only one of the two cohorts, with no significant change in mRNA expression. However, both *PGC-1α* and *PDK4* were

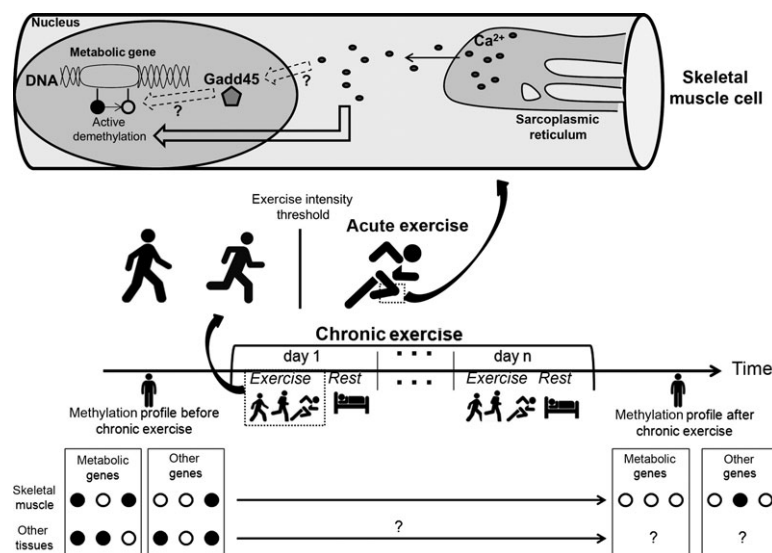


Figure 1 Physical activity influences DNA methylation. The methylation profile of an individual changes in response to physical activity, in a dose-dependent, gene-specific and tissue-specific manner. In skeletal muscle, long-term (chronic) exercise mostly leads to a decrease in the methylation level of metabolic genes such as *PGC-1α* (represented by a shift from a filled circle to an empty circle). Chronic exercise also causes the hypomethylation or the hypermethylation of other genes. During acute exercise, an intensity threshold of effort must be reached to significantly cause a drop in the methylation level of metabolic genes. When the exercise intensity threshold is reached, the release of Ca²⁺ from the sarcoplasmic reticulum, along with other unknown factors, leads to the active demethylation of metabolic genes. Gadd45 is hypothesized to be involved in this active demethylation process. The impact of chronic and acute exercise on other tissues is not well established.

hypomethylated immediately after exercise and strongly transcribed 3 h after (Barrès *et al.* 2012). Interestingly, an earlier study showed that the resting skeletal muscle of diabetic patients has a lower methylation in the promoter of *PDK4*, as measured by direct sequencing, and a 70% higher *PDK4* mRNA level than non-diabetics, probably as a consequence of hyperinsulinaemia and insulin resistance. Furthermore, *PDK4* mRNA expression was increased in response to chronic exercise in healthy participants only, but not in diabetics (Kulkarni *et al.* 2012). The metabolic genes *TFAM* and *PPAR- δ* were also hypomethylated after exercise, but, in contrast to *PGC-1 α* and *PDK4*, their mRNA expression increased immediately after exercise (Barrès *et al.* 2012). Thus, there seems to be variability in the timing of DNA hypomethylation induced by high-intensity exercise and the corresponding gene transcription. Finally, it should also be noted that low-intensity exercise was not sufficient to alter DNA methylation of any of the investigated genes (Barrès *et al.* 2012), suggesting a dose-dependent impact of acute exercise on DNA methylation. Indeed, muscle metabolic profile and fibre type outcome may share common epigenetic mechanisms depending upon the intensity and duration of the exercise stimulus (Barreiro & Sznajder 2013). It is also worth mentioning that DNA methylation might change transiently, before returning back to normal.

DNA methylation changes following chronic exercise training

Gene-specific methylation changes following chronic exercise training. One randomized controlled trial (RCT) and two studies have investigated the influence of chronic exercise training on the methylation status of candidate genes (Alibegovic *et al.* 2010, Nakajima *et al.* 2010, Bryan *et al.* 2012). The RCT examined the methylation status of *p15* (cyclin-dependent kinase inhibitor 2B, a tumour-suppressor gene) and *ASC* (*PYD* and *CARD* domain containing a mediator in the cytosol-type inflammatory signalling pathway), measured by pyrosequencing, in of a group of healthy people whose physical activity was monitored by accelerometry (Nakajima *et al.* 2010). Participants were randomly divided into three groups: a young control group ($n = 34$), an older control group ($n = 153$) and an older training group ($n = 230$) assigned to a 6-month, high-intensity interval walking regimen (3 min of low-intensity walking at 40% of peak aerobic capacity followed by a 3 min of high-intensity walking period above 70% of peak aerobic capacity). Comparison of the control and intervention groups showed no significant correlation between

exercise and the methylation status of *p15* in blood after intervention, but the mean methylation value of the 7 CpG sites tested for *ASC* was significantly greater in the exercise group ($6.3 \pm 0.3\%$) than in the control group ($5.3 \pm 0.1\%$) after intervention. The authors concluded that chronic, moderate-intensity exercise can increase *ASC* methylation and thereby may suppress excess pro-inflammatory cytokine expression. However, mRNA or protein expression was not assessed, making it difficult to draw a definitive conclusion with regard to the functional impact of this very small exercise-induced change in DNA methylation. Another limitation of this study is that no within-group differences from pre- to post-training were measured (Nakajima *et al.* 2010). Thus, it cannot be excluded that, despite randomization and good sample size, other factors impacting the methylation status of the studied CpG sites were not unevenly distributed between the two groups and that the basal level of methylation between the two groups was not already significantly different before study commencement. For instance, the influence of genotype on patterns of DNA methylation is widespread in the genome, and overall genotype can explain 80% of the variation in DNA methylation (Gertz *et al.* 2011). Interestingly, one of the aforementioned observational studies found that exercise-induced DNA methylation changes may be highly dependent on the individual's genotype (Lott *et al.* 2012).

The second study measured, by direct sequencing, the skeletal muscle methylation changes in *PGC-1 α* following an intervention in a cohort of 20 young healthy men. Nine consecutive days of bed rest significantly increased the methylation level of one CpG site by 39% in the promoter of *PGC-1 α* , and this correlated with a decrease in *PGC-1 α* mRNA levels (Alibegovic *et al.* 2010). Then, after a 4-week retraining programme following the bed rest period (supervised cycle ergometry 30 min per day, 6 days per week, at 70% of participants'), there was a tendency towards reversibility of the altered methylation, but it was not significant. Moreover, *PGC-1 α* 's methylation level was no longer significantly correlated with its mRNA expression.

The third study followed a cohort of physically inactive men and women during a 12-month-long exercise promotion intervention (Bryan *et al.* 2012). Despite the fact that the authors used the Infinium HumanMethylation27 BeadChip microarray that interrogates methylation levels genomewide, they chose to focus on the average methylation of 45 CpG sites across 27 genes related to breast cancer, in saliva before and after intervention. At baseline, there was a weak yet significant negative correlation between gene-specific methylation and $VO_{2\max}$ ($r = -0.31$), but not with self-reported physical activity. After the

intervention, gene-specific methylation displayed a small increase (on average, an increase of 1.2%), and so did self-reported physical activity and VO_{2max} . Yet surprisingly, increasing self-reported activity was significantly associated with a decrease in gene-specific methylation. Besides, there was no association between changes in VO_{2max} and changes in gene-specific methylation. Several limitations of this study need to be acknowledged: the sample size was quite small ($n = 64$), DNA methylation changes related to breast cancer were measured in saliva, and there was very little control over duration and intensity of exercise. Indeed, there was considerable variability in the change in self-reported physical activity (average change = 61.98 ± 109.80 min per week). Therefore, the results of this study need to be interpreted with great caution.

Global and genomewide methylation changes following chronic exercise training. While using candidate genes to study the influence of the effect of physical activity on DNA methylation is a valid approach, this requires an *a priori* hypothesis about which genes may be subject to exercise-induced methylation. In order to form this hypothesis, it is necessary to have an understanding about genotype–phenotype relationships (Akey 2009). Additionally, when the individual contribution of multiple genes to the phenotype is small, and the genetic architecture of phenotypic variation remains enigmatic (e.g. elite power and sprint performance; Eynon *et al.* 2013), the ability to nominate candidate genes is constrained (Akey 2009). Thus, candidate gene approaches can sometimes fail to yield validated gene targets (Bouchard 2011). Genomewide explorations can generate much more convincing data and allow understanding of the related mechanisms. To date, three studies have employed the genomewide approach and tested the influence of exercise training on the methylation level of almost all known genes (Zeng *et al.* 2011, Nitert *et al.* 2012, Rönn *et al.* 2013).

The most recent genomewide study compared the average methylation level of each gene in the skeletal muscle from 28 healthy young sedentary individuals, before and after a 6-month exercise training intervention, using MeDIP (Nitert *et al.* 2012). 134 genes showed a significant change in methylation after the exercise intervention, 86% of which were hypomethylated. Genes in which a decreased methylation was observed included *RUNX1* and *MEF2A*, two key transcription factors involved in exercise training (Smith *et al.* 2007, 2008, Keller *et al.* 2011); *NDUFC2*, a part of the respiratory chain (Olsson *et al.* 2011); *ADIPOR1* and *ADIPOR2*, two receptors for adiponectin; *BDKRB2*, a receptor for bradykinin (Taguchi

et al. 2000). For *ADIPOR1* and *BDKRB2*, the decrease in DNA methylation was coupled with an increase in mRNA expression (Nitert *et al.* 2012). This study further identified four biological pathways of genes that had both their methylation altered by exercise and exhibited increased mean transcription values after exercise (purine metabolism, insulin signalling, ErbB signalling and progesterone-mediated oocyte maturation). The same group has also compared genomewide methylation in adipose tissue, using the Infinium HumanMethylation450 BeadChip microarray, in 23 of the 28 participants following 6 months of exercise training (Rönn *et al.* 2013). There was a methylation change in 17975 individual CpG sites corresponding to 7663 unique genes, with most sites (92%) showing increased levels of DNA methylation. For 1/3 of the genes showing a change in DNA methylation, there was also a change in mRNA expression. Notably, these genes included *RALBP1*, *HDAC4* and *NCOR2*. Furthermore, CpG sites of 18 obesity and 21 type 2 diabetes candidate genes respond differently to exercise in adipose tissue, including *TCF7L2* (6 CpG sites) and *KCNQ1* (10 CpG sites).

It is interesting to notice that in muscle, most genes were hypomethylated after intervention (Nitert *et al.* 2012); while in adipose tissue, most genes were hypermethylated after intervention (Rönn *et al.* 2013). In the study conducted in saliva, global DNA methylation slightly increased, as estimated by averaging all sites on the Infinium HumanMethylation27 BeadChip microarray (Bryan *et al.* 2012). This is in contrast with results obtained in blood, for a RCT that included 298 women who underwent a 1-year physical and diet intervention, which did not show any significant change in global DNA methylation measured by pyrosequencing of LINE-1 (Duggan *et al.* 2014). These differences could stem from the investigated tissue where exercise is likely to have distinct impacts. It is also possible that these differences come from the DNA methylation methods used in these studies: in MeDIP, the antibody binding methylated DNA tends to recognize the most densely methylated DNA sequences such as repetitive elements and methylated CpG islands, thus leaving out regions with low methylation (Tost 2008). On the contrary, the Infinium HumanMethylation27 BeadChip mostly investigates promoters regardless of their methylation status, while its expansion the HumanMethylation450 BeadChip also targets gene bodies and intergenic regions that have higher methylation.

The limitation of not including a control group in some studies was addressed in the only other genomewide study to date. A sample of 12 patients with breast cancer were divided into two groups of equal size (one control group and one group undergoing a

6-month-long exercise training intervention; Zeng *et al.* 2011). The change in methylation was measured by the Infinium HumanMethylation27 BeadChip microarray in blood before and after the intervention for every gene and for each participant separately. Then, the average methylation change for each gene was compared between the two groups. This approach allows the observation of DNA methylation changes occurring naturally in a group of patients when regular physical activity is maintained (without exercise training intervention). Forty-three genes showed a small (<8%) yet significant change in DNA methylation level following the exercise intervention, 19 of which were hypomethylated. *GLUD1*, a mitochondrial matrix enzyme regulating energy metabolism (Plaitakis & Zaganas 2001), showed a 5% reduction in methylation in the exercised group compared to the control group. *L3MBTL1*, a known tumour-suppressor gene (Hock *et al.* 2007), and *MSX1*, a gene involved in mammary gland development (Satoh *et al.* 2004), showed a net difference of 3.6% and 4.8%, respectively, in methylation between the two groups. It was further observed using 30 tumour samples from another group of patients that *L3MBTL1* methylation was inversely correlated with *L3MBTL1* transcription ($r = -0.19$) and that high expression of *L3MBTL1* was associated with low risk of breast cancer recurrence and death. These findings suggest that exercise may reduce DNA methylation in *L3MBTL1*, resulting in increased expression of *L3MBTL1*, which may inhibit tumour progression and improve survival.

Summary

Interventional studies seem to agree that exercise significantly impacts the methylation of many genes, including those involved in metabolism and fuel usage (Alibegovic *et al.* 2010, Zeng *et al.* 2011, Barrès *et al.* 2012, Kulkarni *et al.* 2012, Nitert *et al.* 2012, Rönn *et al.* 2013). However, there was no overlap between the identified genes in the three genomewide studies. This can stem from the fact that all studies investigated a different tissue (muscle, adipose and blood). Also, the differences in DNA methylation assays may influence the results. For example, cancer genes are heavily over-represented in the 27k bead chip from Illumina. Thus, the lack of correlation between studies could be a result of gene choice.

In studies using the Infinium HumanMethylation27 BeadChip microarray (Zeng *et al.* 2011, Bryan *et al.* 2012), the inherent technical variability of the Infinium measurements was not taken into consideration. Indeed, it has been demonstrated that with this array, technical replicates can frequently display methylation differences of up to 10% (Dedeurwaerder *et al.*

2013), which is greater than all the DNA methylation changes observed in these studies. Thus, it has been strongly suggested to estimate the intrinsic variability of each probe by running technical replicates, or to use an absolute methylation difference threshold to ensure that the observed DNA methylation changes are not purely artifactual. This step was not omitted by Rönn *et al.* (2013) who filtered out all the CpG sites displaying a methylation change $\leq 5\%$ before vs. after intervention.

Finally, in almost all studies, exercise-induced DNA methylation changes did not always correlate with the expected change in gene expression (Alibegovic *et al.* 2010, Zeng *et al.* 2011, Barrès *et al.* 2012, Nitert *et al.* 2012, Rönn *et al.* 2013). Future research is required to determine whether other epigenetic modifications are involved.

Insights into the molecular mechanisms of exercise-induced DNA methylation changes

DNA methylation is not the sole epigenetic modification involved in exercise

Promoter methylation is generally associated with a repressed chromatin state and inhibition of promoter activity, while gene-body methylation seems to correlate positively with transcription (Bird 2002, Jjingo *et al.* 2012). However, observations from some of the RCTs seem to support the idea that DNA hypomethylation of a promoter is not always necessary for gene transcription (Alibegovic *et al.* 2010, Zeng *et al.* 2011, Barrès *et al.* 2012, Nitert *et al.* 2012, Rönn *et al.* 2013). First, low-intensity exercise was sufficient to increase *PGC-1 α* expression without any significant change in its methylation level (Barrès *et al.* 2012). Second, 2 days following a 3-week exercise training programme, the methylation of *PGC-1 α* remained unaltered, whereas there was an increase in mRNA content (Alibegovic *et al.* 2010). A modest positive correlation was found between the methylation of one CpG site in the *PGC-1 α* promoter and *PGC-1 α* RNA expression ($r = 0.51$) following 4 weeks of retraining after 9 days of bed rest. Similarly, the transcription levels of twenty-three genes did not correlate negatively with their significant DNA methylation change (Nitert *et al.* 2012).

There are other epigenetic modifications that can regulate gene expression. It has also been shown that exercise causes *histone* modifications (Pothhoff *et al.* 2007, McGee *et al.* 2009, Baar 2010) and changes in micro RNA (miRNA) expression (Nielsen *et al.* 2010, Davidsen *et al.* 2011) regulating muscle fibre type, muscle regeneration and muscle mass. Thus, the presence of transcription factors and/or miRNAs,

as well as histone modifications, may impact gene expression to such an extent that the effect of DNA methylation becomes minor. For instance, the lack of correlation between *PGC-1 α* methylation and its mRNA level (Alibegovic *et al.* 2010, Barrès *et al.* 2012) may be due to the influence of transcription factors, such as cAMP response element-binding protein (CREB) and Parkin-interacting substrate (PARIS), known to control *PGC-1 α* expression (Lindholm *et al.* 2012). Another factor could be the miRNA miR-696 which was recently shown to regulate *PGC-1 α* expression in mouse skeletal muscle in response to physical exercise (Aoi *et al.* 2010). These factors may also account for the variable time lapse observed between the decrease in methylation and increased expression of different genes (Barrès *et al.* 2012).

Exercise and DNA hypomethylation

Although in most cases DNA methylation is a stable epigenetic mark, reduced levels of methylation are observed during development in both plants and mammals (Law & Jacobsen 2010). Demethylation can be achieved passively, in the course of cell division, or actively through mechanisms that are only starting to be understood (Bhutani *et al.* 2011). The passive pathway is rather slow and involves the hydroxylation of methylcytosines, which impairs remethylation by DNMTs (DNA methyltransferases). In contrast, active loss of methylation occurs rapidly and is independent of DNA replication (Bhutani *et al.* 2011). In the case of acute exercise, DNA hypomethylation seems to happen quickly and to be only transient (Barrès *et al.* 2012) and is therefore likely to involve active demethylation pathways.

One proposed mechanism for active demethylation involves ten-eleven translocation (TET; Tahiliani *et al.* 2009) as reviewed in (Bhutani *et al.* 2011). In this pathway, hydroxymethylcytosines are an intermediate for demethylation. Pareja-Galeano *et al.* (2014) suggested that metabolites produced during exercise could alter the activity of TET proteins, thus inducing DNA demethylation. However, the bisulphite modification technique used in the acute exercise intervention study (Barrès *et al.* 2012) does not allow discrimination between methyl and hydroxymethylcytosine (Tahiliani *et al.* 2009). Thus, the contribution of the TET pathway to exercise-induced demethylation remains to be determined (Rasmussen *et al.* 2014).

There are two other demethylation circuitries that do not involve the hydroxylation of methylcytosines: the first one consists of the deamination of 5meC to T followed by base excision repair (BER), and the

second one is achieved through nucleotide excision repair (NER). In the first pathway, deamination can be achieved thanks to activation-induced deaminase (AID), and the mismatch is repaired by T DNA glycosylase (TDG) or by methyl-CpG-binding protein (MBD4). Also, DNA methyl transferases 3A and 3B have recently been discovered to possess deaminase activity, but the biochemical sequence is presently unknown (Kangaspeska *et al.* 2008, Métivier *et al.* 2008). AID, TDG and MBD4 all act cooperatively with Gadd45 (Rai *et al.* 2008, Niehrs & Schäfer 2012). In the second pathway, Gadd45a interacts with the NER xeroderma pigmentosum group G-complementing protein (XPG). Interestingly, highly active women who had a specific genotype in XPG experienced significant breast cancer risk reductions compared to inactive women with the same genotype (McCullough *et al.* 2013). Besides, Gadd45a-mediated DNA demethylation seems to be gene specific in mammalian cells (Jin *et al.* 2008, Engel *et al.* 2009, Schäfer *et al.* 2010), which is consistent with the gene-specific, exercise-induced hypomethylation observed in the previous studies. Moreover, Gadd45 has been shown to be upregulated after a strenuous physical load in amateur runners (Marfe *et al.* 2010), and Gadd45a and Gadd45b are upregulated after calcium treatment (Sen *et al.* 2010). Interestingly, it has been shown in an *ex vivo* model of muscle contraction that exercise-induced disturbances in intracellular homeostasis are sufficient to induce DNA methylation remodelling, regardless of any change in neurotransmitters or circulating factors (Barrès *et al.* 2012). Specifically, it was found that cytoplasmic calcium release is necessary (yet not sufficient) to induce DNA hypomethylation, but neither changes in the adenosine mono phosphate (AMP): ATP ratio nor the intracellular redox state is involved in promoter hypomethylation. This observation is in contrast with the established impact of exercise on hormone release (e.g. growth hormone, insulin-like growth factor 1 and steroids; Robergs & Roberts 1997) and subsequent histone modifications (Collins *et al.* 2009, Chia *et al.* 2010). For that matter, doping is suspected to influence epigenetic processes (Schwarzenbach 2011). Thus, exercise seems to impact epigenetic mechanisms via two different pathways: one hormone independent and one hormone dependent. Finally, neurones lacking Gadd45b fail to demethylate DNA and thereby fail to activate brain-derived neurotrophic factor (*BDNF*; Ma *et al.* 2009, Guo *et al.* 2011). Exercise causes the hypomethylation of *BDNF* in rats (Gomez-Pinilla *et al.* 2011), further suggesting an implication of Gadd45-mediated demethylation in exercise-induced hypomethylation. However, at this point, the implication of Gadd45 in exercise-induced demethylation requires experimental

verification. Moreover, it is possible that chronic exercise impacts DNA methylation through a different mechanism than acute exercise.

Conclusions

While observational studies report weak correlation between physical activity and DNA methylation, interventional studies have systematically identified a significant impact of acute or chronic exercise on DNA methylation in different tissues (Fig. 1). Among the genes whose methylation levels were found to be significantly changed after exercise were genes involved in metabolism (including *PGC-1 α* , *GLUD1*, *PDK-4*, *PPAR- δ* , *TFAM*, *ADIPOR1*, *ADIPOR2* and *BDKRB2*), muscle growth (*MEF2A*), haematopoiesis (*RUNX1*) and inflammation (*ASC*). The lack of a significant correlation in some observational studies could have three explanations. First, it is possible that the limitations of observational studies (e.g. selection bias, recall errors from the participants) have made it difficult to identify a correlation between chronic physical activity and DNA methylation. Second, one consistent finding across all interventional studies is that acute or chronic exercise impacts DNA methylation in a gene-specific manner. Therefore, it is possible that the studies unable to find a significant correlation between physical activity and DNA methylation have targeted genes unaffected by physical activity. However, an interesting observation is that all studies reporting negative results were focused on cancer. Thus, it is also possible that physical activity has a rather negligible impact on cancer-related DNA methylation. Another explanation could be that cancer research usually reports an on or off effect with DNA methylation changing from 0 to 100%, whereas exercise might have multiple more subtle changes. It is possible that the changes are there, but are excluded as too small to be quantified. Third, it should be noted that in each study, DNA methylation was always assessed in a single tissue; thus, it cannot be excluded that DNA methylation changes initiated by physical activity occurred in other non-tested tissues.

The impact of acute exercise on DNA methylation seems to depend on the intensity of the exercise. Also, a change in DNA methylation caused by exercise does not always correlate with a change in mRNA expression in opposite directions for all impacted genes. The molecular pathway through which exercise causes these DNA methylation changes remains unknown. It seems that cytoplasmic calcium release is necessary yet not sufficient to achieve active DNA demethylation. However, circulating factors and neurotransmitters do not seem to be involved in active DNA demethylation. There are several possible ways to achieve active

DNA demethylation: by oxidative demethylation, by deamination of 5mC to T followed by BER, and by NER. Among them, the pathways involving Gadd45 appear to be the best candidates, but there is insufficient evidence to rule out other pathways.

Recommendations for future research

As previously mentioned, the study design is important to examine a possible link between physical activity and DNA methylation without bias, and RCTs seem to be the best among all the studies. If the purpose of a study is to examine the impact of a chronic exercise intervention on DNA methylation, the presence of a control group is important to account for the DNA methylation changes that may occur independently of the exercise training intervention. Exercise-induced DNA methylation is not always inversely correlated with mRNA levels. Furthermore, DNA methylation changes and gene expression seem to happen at variable rates after acute exercise. All of these observations highlight the complexity of the relationship between DNA methylation and transcription, and it is a good reminder that gene expression, and probably also protein changes, should systematically be assessed along with DNA methylation. Finally, it is unknown to what extent the genetic background of an individual impacts exercise-induced DNA methylation changes. Thus, ethnic stratification or stratification based on specific genotypes of interest is important prior data analysis.

DNA methylation changes caused by acute exercise seem to depend on the intensity of the exercise, but little is known about the effect of the duration of exercise. The impact of chronic exercise on DNA methylation may be different from the impact of acute exercise on DNA methylation, and in this regard, more acute studies need to be conducted. Also, chronic physical activity over a long period of time (years or a lifetime) may have a different impact on DNA methylation than physical activity over shorter periods of time (weeks or months). Whether different durations of physical activity target the same tissues and the same genomic sites is unknown. It is also unknown whether DNA methylation changes are transient or stable over time. Thus, it may be worth comparing the rates and genomic locations of DNA methylation changes induced by physical activity of different durations. For instance, a good study design could be a long-term follow-up study on a cohort whose changes in lifestyle (e.g. physical activity) are monitored, and whose DNA methylation levels are regularly assessed in relevant tissues such as adipose tissue and skeletal muscle. Another interesting study design would involve periods of training and

detraining to explore the stability of exercise-induced DNA methylation changes.

So far, almost all studies have focused on the effect of aerobic exercise on DNA methylation. However, anaerobic exercise has a different impact on cell homeostasis, and it is unknown whether aerobic exercise and anaerobic exercise impact DNA methylation in a similar way. Future studies should compare the effects of these two types of exercise on DNA methylation.

DNA methylation patterns are known to vary between tissues, and even in a cell-specific manner within some tissues. The specific impact of physical activity on the methylation pattern of the different tissues in the human body is unknown. The differences, but also the similarities between tissues with regard to exercise-induced DNA methylation changes, should be assessed. There may be methylation changes, but it is unknown whether they are correlated with the health benefits provided by physical activity.

More studies are needed to uncover the molecular mechanism through which exercise causes active DNA demethylation, with a special focus on pathways involving Gadd45. Exercise causes a decrease in the methylation of many genes, but there are some genes whose methylation levels are increased by exercise. How does physical activity cause the hypomethylation of some genes and the hypermethylation of other genes at the same time?

Finally, future work is needed to determine to what extent epigenetic patterns impact on athletic performance and trainability. Exercise training might favourably shape the epigenome of an athlete towards higher performance, but there is not enough data to support this hypothesis. However, if this turned out to be the case, it would be interesting to measure the extent to which these epigenetic changes are passed on to the next generation. It would also be fascinating to see whether these epigenetic changes are stable or reversed if exercise training is interrupted.

Conflicts of interest

There are no conflict of interests for the article.

References

Adeyemo, A., Gerry, N., Chen, G., Herbert, A., Doumatey, A., Huang, H., Zhou, J., Lashley, K., Chen, Y., Christman, M. & Rotimi, C. 2009. A genome-wide association study of hypertension and blood pressure in African Americans. *PLoS Genet* 5, 11.

Akey, J.M. 2009. Constructing genomic maps of positive selection in humans: where do we go from here? *Genome Res* 19, 711–722.

Alibegovic, A.C., Sonne, M.P., Højbjerg, L., Bork-Jensen, J., Jacobsen, S., Nilsson, E., Faerch, K., Hiscock, N., Mortensen, B., Friedrichsen, M., Stallknecht, B., Dela, F. & Vaag, A. 2010. Insulin resistance induced by physical inactivity is associated with multiple transcriptional changes in skeletal muscle in young men. *Am J Physiol Endocrinol Metab* 299, E752–E763.

An, P., Pérusse, L., Rankinen, T., Borecki, I.B., Gagnon, J., Leon, A.S., Skinner, J.S., Wilmore, J.H., Bouchard, C. & Rao, D.C. 2003. Familial aggregation of exercise heart rate and blood pressure in response to 20 weeks of endurance training: the HERITAGE family study. *Int J Sports Med* 24, 57–62.

Aoi, W., Naito, Y., Mizushima, K., Takanami, Y., Kawai, Y., Ichikawa, H. & Yoshikawa, T. 2010. The microRNA miR-696 regulates PGC-1 α in mouse skeletal muscle in response to physical activity. *Am J Physiol Endocrinol Metab* 298, E799–E806.

Baar, K. 2010. Epigenetic control of skeletal muscle fibre type. *Acta Physiol (Oxf)* 199, 477–487.

Ballard-Barbash, R., Friedenreich, C.M., Courneya, K.S., Siddiqi, S.M., McTiernan, A. & Alfano, C.M. 2012. Physical activity, biomarkers, and disease outcomes in cancer survivors: a systematic review. *J Natl Cancer Inst* 104, 815–840.

Barreiro, E. & Sznajder, J.I. 2013. Epigenetic regulation of muscle phenotype and adaptation: a potential role in COPD muscle dysfunction. *J Appl Physiol (1985)* 114, 1263–1272.

Barrès, R., Yan, J., Egan, B., Treebak Jonas, T., Rasmussen, M., Fritz, T., Caidahl, K., Krook, A., O’Gorman Donal, J. & Zierath Juleen, R. 2012. Acute exercise remodels promoter methylation in human skeletal muscle. *Cell Metab* 15, 405–411.

Baylin, S.B. & Jones, P.A. 2011. A decade of exploring the cancer epigenome — biological and translational implications. *Nat Rev Cancer* 11, 726–734.

Berman, Y. & North, K.N. 2010. A gene for speed: the emerging role of α -actinin-3 in muscle metabolism. *Physiology* 25, 250–259.

Bhutani, N., Burns, D.M. & Blau, H.M. 2011. DNA demethylation dynamics. *Cell* 146, 866–872.

Bird, A. 2002. DNA methylation patterns and epigenetic memory. *Genes Dev* 16, 6–21.

Bishop, D.J., Thomas, C., Moore-Morris, T., Tonkonogi, M., Sahlin, K. & Mercier, J. 2010. Sodium bicarbonate ingestion prior to training improves mitochondrial adaptations in rats. *Am J Physiol Endocrinol Metab* 299, E225–E233.

Bishop, D.J., Granata, C. & Eynon, N. 2013. Can we optimise the exercise training prescription to maximise improvements in mitochondria function and content? *Biochim Biophys Acta* 1840, 1266–1275.

Bollati, V. & Baccarelli, A. 2010. Environmental epigenetics. *Heredity* 105, 105–112.

Bonasio, R., Tu, S. & Reinberg, D. 2010. Molecular signals of epigenetic states. *Science* 330, 612–616.

Bouchard, C. 2011. Genomic predictors of trainability (genomics & exercise). *Exp Physiol* 3, 347–352.

Bouchard, C., Leon, A.S., Rao, D.C., Skinner, J.S., Wilmore, J.H. & Gagnon, J. 1995. The HERITAGE family study.

- Aims, design, and measurement protocol. *Med Sci Sports Exerc* 27, 721–729.
- Bouchard, C., An, P., Rice, T., Skinner, J.S., Wilmore, J.H., Gagnon, J., Pérusse, L., Leon, A.S. & Rao, D.C. 1999. Familial aggregation of $\text{VO}_{2\text{max}}$ response to exercise training: results from the HERITAGE Family Study. *J Appl Physiol* 87, 1003–1008.
- Bouchard, C., Sarzynski, M.A., Rice, T.K., Kraus, W.E., Church, T.S., Sung, Y.J., Rao, D.C. & Rankinen, T. 2011. Genomic predictors of the maximal O uptake response to standardized exercise training programs. *J Appl Physiol* (1985) 110, 1160–1170.
- Broos, S., Malisoux, L., Theisen, D., Francaux, M., Deldicque, L. & Thomis, M.A. 2012. Role of alpha-actinin-3 in contractile properties of human single muscle fibers: a case series study in paraplegics. *PLoS ONE* 7, e49281.
- Bryan, A.D., Magnan, R.E., Hooper, A.E.C., Harlaar, N. & Hutchison, K.E. 2012. Physical activity and differential methylation of breast cancer genes assayed from saliva: a preliminary investigation. *Ann Behav Med* 45, 89–98.
- Chia, D.J., Young, J.J., Mertens, A.R. & Rotwein, P. 2010. Distinct alterations in chromatin organization of the two IGF-I promoters precede growth hormone-induced activation of IGF-I gene transcription. *Mol Endocrinol* 24, 779–789.
- Clarke-Harris, R., Wilkin, T.J., Hosking, J., Pinkney, J., Jeffery, A.N., Metcalf, B.S., Godfrey, K.M., Voss, L.D., Lillycrop, K.A. & Burdge, G.C. 2014. PGC1 α promoter methylation in blood at 5-7 years predicts adiposity from 9 to 14 years (EarlyBird 50). *Diabetes* 63, 2528–2537.
- Collins, A., Hill, L.E., Chandramohan, Y., Whitcomb, D., Droste, S.K. & Reul, J.M.H.M. 2009. Exercise improves cognitive responses to psychological stress through enhancement of epigenetic mechanisms and gene expression in the dentate gyrus. *PLoS ONE* 4, 11.
- Coyle, Y.M., Xie, X.-J., Lewis, C.M., Bu, D., Milchgrub, S. & Euhus, D.M. 2007. Role of physical activity in modulating breast cancer risk as defined by APC and RASSF1A promoter hypermethylation in nonmalignant breast tissue. *Cancer Epidemiol Biomarkers Prev* 16, 192–196.
- Davidson, P.K., Gallagher, I.J., Hartman, J.W., Tarnopolsky, M.A., Dela, F., Helge, J.W., Timmons, J.A. & Phillips, S.M. 2011. High responders to resistance exercise training demonstrate differential regulation of skeletal muscle microRNA expression. *J Appl Physiol* 110, 309–317.
- Dawson, M.A. & Kouzarides, T. 2012. Cancer epigenetics: from mechanism to therapy. *Cell* 150, 12–27.
- Dedeurwaerder, S., Defrance, M., Bizet, M., Calonne, E., Bontempi, G. & Fuks, F. 2013. A comprehensive overview of Infinium HumanMethylation450 data processing. *Brief Bioinform* 2013, bbt054.
- Denham, J., Marques, F.Z., O'Brien, B.J. & Charchar, F.J. 2013. Exercise: putting action into our epigenome. *Sports Med* 44, 189–209.
- Duggan, C., Xiao, L., Terry, M.B. & McTiernan, A. 2014. No effect of weight loss on LINE-1 methylation levels in peripheral blood leukocytes from postmenopausal overweight women. *Obesity* 22, 2091–2096.
- Ehlert, T., Simon, P. & Moser, D.A. 2013. Epigenetics in sports. *Sports Med* 43, 93–110.
- Ehrlich, M. 2002. DNA methylation in cancer: too much, but also too little. *Oncogene* 21, 5400–5413.
- Engel, N., Tront, J.S., Erinle, T., Nguyen, N., Latham, K.E., Sapienza, C., Hoffman, B. & Liebermann, D.A. 2009. Conserved DNA methylation in Gadd45a(–/–) mice. *Epigenetics* 4, 98–99.
- Eynon, N., Duarte, J.A., Oliveira, J., Sagiv, M., Yamin, C., Meckel, Y. & Goldhammer, E. 2009. ACTN3 R577X polymorphism and Israeli top-level athletes. *Int J Sports Med* 30, 695–698.
- Eynon, N., Ruiz, J.R., Oliveira, J., Duarte, J.A., Birk, R. & Lucia, A. 2011. Genes and elite athletes: a roadmap for future research. *J Physiol* 589, 3063–3070.
- Eynon, N., Ruiz, J.R., Femia, P., Pushkarev, V.P., Cieszczyk, P., Maciejewska-Karłowska, A., Sawczuk, M., Dyatlov, D.A., Lekontsev, E.V., Kulikov, L.M., Birk, R., Bishop, D.J. & Lucia, A. 2012. The ACTN3 R577X polymorphism across three groups of elite male European athletes. *PLoS ONE* 7, e43132.
- Eynon, N., Hanson, E.D., Lucia, A., Houweling, P.J., Garton, F., North, K.N. & Bishop, D.J. 2013. Genes for elite power and sprint performance: ACTN3 leads the way. *Sports Med* 43, 803–817.
- Folland, J.P., Mc Cauley, T.M., Phipers, C., Hanson, B. & Mastana, S.S. 2012. The relationship of testosterone and AR CAG repeat genotype with knee extensor muscle function of young and older men. *Exp Gerontol* 47, 437–443.
- Garatachea, N., Fiuza-Luces, C., Torres-Luque, G., Yvert, T., Santiago, C., Gómez-Gallego, F., Ruiz, J.R. & Lucia, A. 2011. Single and combined influence of ACE and ACTN3 genotypes on muscle phenotypes in octogenarians. *Eur J Appl Physiol* 112, 2409–2420.
- Gertz, J., Varley, K.E., Reddy, T.E., Bowling, K.M., Pauli, F., Parker, S.L., Kucera, K.S., Willard, H.F. & Myers, R.M. 2011. Analysis of DNA methylation in a three-generation family reveals widespread genetic influence on epigenetic regulation. *PLoS Genet* 7, e1002228.
- Gluckman, P.D., Hanson, M.A. & Beedle, A.S. 2007. Non-genomic transgenerational inheritance of disease risk. *Bioessays* 29, 145–154.
- Gomes, M.V.M., Toffoli, L.V., Arruda, D.W., Soldara, L.M., Pelosi, G.G., Neves-Souza, R.D., Freitas, E.R., Castro, D.T. & Marquez, A.S. 2012. Age-related changes in the global DNA methylation profile of leukocytes are linked to nutrition but are not associated with the MTHFR C677T genotype or to functional capacities. *PLoS ONE* 7, e52570.
- Gomez-Pinilla, F., Zhuang, Y., Feng, J., Ying, Z. & Fan, G. 2011. Exercise impacts brain-derived neurotrophic factor plasticity by engaging mechanisms of epigenetic regulation. *Eur J Neurosci* 33, 383–390.
- Guo, J.U., Ma, D.K., Mo, H., Ball, M.P., Jang, M.-H., Bonaguidi, M.A., Balazer, J.A., Eaves, H.L., Xie, B., Ford, E., Zhang, K., Ming, G.-L., Gao, Y. & Song, H. 2011. Neuronal activity modifies the DNA methylation landscape in the adult brain. *Nat Neurosci* 14, 1345–1351.

- Hannan, E.L. 2008. Randomized clinical trials and observational studies: guidelines for assessing respective strengths and limitations. *JACC Cardiovasc Interv* 1, 211–217.
- Hashimshony, T., Zhang, J., Keshet, I., Bustin, M. & Cedar, H. 2003. The role of DNA methylation in setting up chromatin structure during development. *Nat Genet* 34, 187–192.
- Hock, R., Furusawa, T., Ueda, T. & Bustin, M. 2007. MBT domain proteins in development and disease. *Trends Cell Biol* 21, 221–230.
- Ingrosso, D. & Perna, A.F. 2009. Epigenetics in hyperhomocysteinemic states. A special focus on uremia. *Biochim Biophys Acta* 1790, 892–899.
- Joung, N.H. & Harris, R.A. 2008. Pyruvate dehydrogenase kinase-4 deficiency lowers blood glucose and improves glucose tolerance in diet-induced obese mice. *Am J Physiol Endocrinol Metab* 295, E46–E54.
- Jepsen, P., Johnsen, S.P., Gillman, M.W. & Sørensen, H.T. 2004. Interpretation of observational studies. *Heart* 90, 956–960.
- Jin, S.-G., Guo, C. & Pfeifer, G.P. 2008. GADD45A does not promote DNA demethylation. *PLoS Genet* 4, 9.
- Jirtle, R.L. & Skinner, M.K. 2007. Environmental epigenomics and disease susceptibility. *Nat Rev Genet* 8, 253–262.
- Jjingo, D., Conley, A.B., Yi, S.V., Lunyak, V.V. & Jordan, I.K. 2012. On the presence and role of human gene-body DNA methylation. *Oncotarget* 3, 462–474.
- Kangaspeska, S., Stride, B., Métivier, R., Polycarpou-Schwarz, M., Ibberson, D., Carmouche, R.P., Benes, V., Gannon, F. & Reid, G. 2008. Transient cyclical methylation of promoter DNA. *Nature* 452, 112–115.
- Keller, P., Vollaard, N.B.J., Gustafsson, T., Gallagher, I.J., Sundberg, C.J., Rankinen, T., Britton, S.L., Bouchard, C., Koch, L.G. & Timmons, J.A. 2011. A transcriptional map of the impact of endurance exercise training on skeletal muscle phenotype. *J Appl Physiol* 110, 46–59.
- Kulkarni, S.S., Salehzadeh, F., Fritz, T., Zierath, J.R., Krook, A. & Osler, M.E. 2012. Mitochondrial regulators of fatty acid metabolism reflect metabolic dysfunction in type 2 diabetes mellitus. *Metab Clin Exp* 61, 175–185.
- Law, J.A. & Jacobsen, S.E. 2010. Establishing, maintaining and modifying DNA methylation patterns in plants and animals. *Nat Rev Genet* 11, 204–220.
- Lindholm, D., Eriksson, O., Mäkelä, J., Belluardo, N. & Korhonen, L. 2012. PGC-1 α : a master gene that is hard to master. *Cell Mol Life Sci* 69, 2465–2468.
- Lira, V.A., Benton, C.R., Yan, Z. & Bonen, A. 2010. PGC-1 α regulation by exercise training and its influences on muscle function and insulin sensitivity. *Am J Physiol Endocrinol Metab* 299, E145–E161.
- Lissner, L., Potischman, N., Troiano, R. & Bengtsson, C. 2004. Recall of physical activity in the distant past: the 32-year follow-up of the Prospective Population Study of Women in Göteborg, Sweden. *Am J Epidemiol* 159, 304–307.
- Little, J.P., Safdar, A., Bishop, D., Tarnopolsky, M.A. & Gibala, M.J. 2011. An acute bout of high-intensity interval training increases the nuclear abundance of PGC-1 α and activates mitochondrial biogenesis in human skeletal muscle. *Am J Physiol Regul Integr Comp Physiol* 300, R1303–R1310.
- Lott, S.A., Burghardt, P.R., Burghardt, K.J., Bly, M.J., Grove, T.B. & Ellingrod, V.L. 2012. The influence of metabolic syndrome, physical activity and genotype on catechol-O-methyl transferase promoter-region methylation in schizophrenia. *Pharmacogenomics J* 13, 264–271.
- Luttrupp, K., Nordfors, L., Ekström, T. & Lind, L. 2013. Physical activity is associated with decreased global DNA methylation in Swedish older individuals. *Scand J Clin Lab Invest* 73, 184–185.
- Ma, D.K., Jang, M.H., Guo, J.U., Kitabatake, Y., Chang, M.L., Pow-Anpongkul, N., Flavell, R.A., Lu, B., Ming, G.L. & Song, H. 2009. Neuronal activity-induced Gadd45b promotes epigenetic DNA demethylation and adult neurogenesis. *Science* 323, 1074–1077.
- Marfe, G., Tafani, M., Pucci, B., Di Stefano, C., Indelicato, M., Andreoli, A., Russo, M.A., Sinibaldi-Salimei, P. & Manzi, V. 2010. The effect of marathon on mRNA expression of anti-apoptotic and pro-apoptotic proteins and sirtuins family in male recreational long-distance runners. *BMC Physiol* 10, 7.
- Matthews, C. 2002. Use of self-report instruments to assess physical activity. In: G. Welk (ed.) *Physical Activity Assessments for Health Related Research*, pp. 107–123. Human Kinetics, Champaign, IL.
- McCullough, L.E., Santella, R.M., Cleveland, R.J., Millikan, R.C., Olshan, A.F., North, K.E., Bradshaw, P.T., Eng, S.M., Terry, M.B., Shen, J., Crew, K.D., Rossner, P., Teitelbaum, S.L., Neugut, A.I. & Gammon, M.D. 2013. Polymorphisms in DNA repair genes, recreational physical activity and breast cancer risk. *Int J Cancer* 134, 654–663.
- McGee, S.L., Fairlie, E., Garnham, A.P. & Hargreaves, M. 2009. Exercise-induced histone modifications in human skeletal muscle. *J Physiol* 587, 5951–5958.
- Métivier, R., Gallais, R., Tiffocche, C., Le Péron, C., Jurkowska, R.Z., Carmouche, R.P., Ibberson, D., Barath, P., Demay, F., Reid, G., Benes, V., Jeltsch, A., Gannon, F. & Salbert, G. 2008. Cyclical DNA methylation of a transcriptionally active promoter. *Nature* 452, 45–50.
- Mitchell, R.J., Farrington, S.M., Dunlop, M.G. & Campbell, H. 2002. Mismatch repair genes hMLH1 and hMSH2 and colorectal cancer: a HuGE review. *Am J Epidemiol* 156, 885–902.
- Morabia, A., Zhang, F.F., Kappil, M.A., Flory, J., Mirer, F.E., Santella, R.M., Wolff, M. & Markowitz, S.B. 2012. Biologic and epigenetic impact of commuting to work by car or using public transportation: a case-control study. *Prev Med* 54, 229–233.
- Nakajima, K., Takeoka, M., Mori, M., Hashimoto, S., Sakurai, A., Nose, H., Higuchi, K., Itano, N., Shiohara, M., Oh, T. & Taniguchi, S. 2010. Exercise effects on methylation of ASC gene. *Int J Sports Med* 31, 671–675.
- Niehrs, C. & Schäfer, A. 2012. Active DNA demethylation by Gadd45 and DNA repair. *Trends Cell Biol* 22, 1–8.

- Nielsen, S., Scheele, C., Yfanti, C., Akerström, T., Nielsen, A.R., Pedersen, B.K. & Laye, M.J. 2010. Muscle specific microRNAs are regulated by endurance exercise in human skeletal muscle. *J Physiol* **588**, 4029–4037.
- Nitert, M.D., Dayeh, T., Volkov, P., Elgzyri, T., Hall, E., Nilsson, E., Yang, B.T., Lang, S., Parikh, H., Wessman, Y. et al. 2012. Impact of an exercise intervention on DNA methylation in skeletal muscle from first-degree relatives of patients with type 2 diabetes. *Diabetes* **61**, 3322–3332.
- Olsson, A.H., Rönn, T., Ladenvall, C., Parikh, H., Isomaa, B., Groop, L. & Ling, C. 2011. Two common genetic variants near nuclear-encoded OXPHOS genes are associated with insulin secretion in vivo. *Eur J Endocrinol* **164**, 765–771.
- Pareja-Galeano, H., Sanchis-Gomar, F. & García-Giménez, J.L. 2014. Physical exercise and epigenetic modulation: elucidating intricate mechanisms. *Sports Med* **44**, 3322–3332.
- Pérusse, L., Gagnon, J., Province, M.A., Rao, D.C., Wilmore, J.H., Leon, A.S., Bouchard, C. & Skinner, J.S. 2001. Familial aggregation of submaximal aerobic performance in the HERITAGE Family study. *Med Sci Sports Exerc* **33**, 597–604.
- Pilegaard, H. & Neufer, P.D. 2004. Transcriptional regulation of pyruvate dehydrogenase kinase 4 in skeletal muscle during and after exercise. *Proc Nutr Soc* **63**, 221–226.
- Pirola, C.J., Fernández Gianotti, T., Burgueño, A.L., Rey-Funes, M., Loidl, C.F., Mallardi, P., San Martino, J., Castaño, G.O. & Sookoian, S. 2012. Epigenetic modification of liver mitochondrial DNA is associated with histological severity of nonalcoholic fatty liver disease. *Gut* **62**, 1356–1363.
- Platakis, A. & Zaganas, I. 2001. Regulation of human glutamate dehydrogenases: implications for glutamate, ammonia and energy metabolism in brain. *J Neurosci Res* **66**, 899–908.
- Potthoff, M.J., Wu, H., Arnold, M.A., Shelton, J.M., Backs, J., McAnally, J., Richardson, J.A., Bassel-Duby, R. & Olson, E.N. 2007. Histone deacetylase degradation and MEF2 activation promote the formation of slow-twitch myofibers. *J Clin Invest* **117**, 2459–2467.
- Puthuchery, Z., Skipworth, J.R.A., Rawal, J., Loosemore, M., Van Someren, K. & Montgomery, H.E. 2011. The ACE gene and human performance: 12 years on. *Sports Med* **41**, 433–448.
- Rai, K., Huggins, I.J., James, S.R., Karpf, A.R., Jones, D.A. & Cairns, B.R. 2008. DNA demethylation in Zebrafish involves the coupling of a deaminase, a glycosylase, and Gadd45. *Cell* **135**, 1201–1212.
- Rasmussen, M., Zierath, J.R. & Barrès, R. 2014. Dynamic epigenetic responses to muscle contraction. *Drug Discov Today* **19**, 1010–1014.
- Reik, W., Dean, W. & Walter, J. 2001. Epigenetic reprogramming in mammalian development. *Science* **293**, 1089–1093.
- Ren, H., Collins, V., Clarke, S.J., Han, J.-S., Lam, P., Clay, F., Williamson, L.M. & Andy Choo, K.H. 2012. Epigenetic changes in response to tai chi practice: a pilot investigation of DNA methylation marks. *Evid Based Complement Alternat Med* **2012**, 841810.
- Roberts, R.A. & Roberts, S.O. 1997. Exercise physiology: exercise, performance, and clinical applications. St. Louis, Mosby.
- Rönn, T., Volkov, P., Davegårdh, C., Dayeh, T., Hall, E., Olsson, A.H., Nilsson, E., Tornberg, A., Dekker Nitert, M., Eriksson, K.-F., Jones, H.a., Groop, L. & Ling, C. 2013. A six months exercise intervention influences the genome-wide DNA methylation pattern in human adipose tissue. *PLoS Genet* **9**, e1003572.
- Sanchis-Gomar, F., Garcia-Gimenez, J.L., Perez-Quilis, C., Gomez-Cabrera, M.C., Pallardo, F.V. & Lippi, G. 2012. Physical exercise as an epigenetic modulator: eustress, the “positive stress” as an effector of gene expression. *J Strength Cond Res* **26**, 3469–3472.
- Satoh, K., Ginsburg, E. & Vonderhaar, B.K. 2004. Msx-1 and Msx-2 in mammary gland development. *J Mammary Gland Biol Neoplasia* **9**, 195–205.
- Schäfer, A., Schomacher, L., Barreto, G., Döderlein, G. & Niehrs, C. 2010. Gemcitabine functions epigenetically by inhibiting repair mediated DNA demethylation. *PLoS ONE* **5**, 9.
- Schwarzenbach, H. 2011. Impact of physical activity and doping on epigenetic gene regulation. *Drug Test Anal* **3**, 682–687.
- Sen, G.L., Reuter, J.A., Webster, D.E., Zhu, L. & Khavari, P.A. 2010. DNMT1 maintains progenitor function in self-renewing somatic tissue. *Nature* **463**, 563–567.
- Sharma, S., Kelly, T.K. & Jones, P.A. 2010. Epigenetics in cancer. *Carcinogenesis* **31**, 27–36.
- Shen, L. & Waterland, R.A. 2007. Methods of DNA methylation analysis. *Curr Opin Clin Nutr Metab Care* **10**, 576–581.
- Shock, L.S., Thakkar, P.V., Peterson, E.J., Moran, R.G. & Taylor, S.M. 2011. DNA methyltransferase 1, cytosine methylation, and cytosine hydroxymethylation in mammalian mitochondria. *Proc Natl Acad Sci USA* **108**, 3630–3635.
- Slattery, M.L., Curtin, K., Sweeney, C., Levin, T.R., Potter, J., Wolff, R.K., Albertsen, H. & Samowitz, W.S. 2007. Diet and lifestyle factor associations with CpG island methylator phenotype and BRAF mutations in colon cancer. *Int J Cancer* **120**, 656–663.
- Smith, J.A.H., Collins, M., Grobler, L.A., Magee, C.J. & Ojuka, E.O. 2007. Exercise and CaMK activation both increase the binding of MEF2A to the Glut4 promoter in skeletal muscle in vivo. *Am J Physiol Endocrinol Metab* **292**, E413–E420.
- Smith, J.A., Kohn, T.A., Chetty, A.K. & Ojuka, E.O. 2008. CaMK activation during exercise is required for histone hyperacetylation and MEF2A binding at the MEF2 site on the Glut4 gene. *Am J Physiol Endocrinol Metab* **295**, E698–E704.
- Smolarek, I., Wyszko, E., Barciszewska, A.M., Nowak, S., Gawronska, I., Jablecka, A. & Barciszewska, M.Z. 2010. Global DNA methylation changes in blood of patients with essential hypertension. *Med Sci Monit* **16**, CR149–CR155.

- Taguchi, T., Kishikawa, H., Motoshima, H., Sakai, K., Nishiyama, T., Yoshizato, K., Shirakami, A., Toyonaga, T., Shirontani, T., Araki, E. & Shichiri, M. 2000. Involvement of bradykinin in acute exercise-induced increase of glucose uptake and GLUT-4 translocation in skeletal muscle: studies in normal and diabetic humans and rats. *Metab, Clin Exp* **49**, 920–930.
- Tahiliani, M., Koh, K.P., Shen, Y., Pastor, W.A., Bandukwala, H., Brudno, Y., Agarwal, S., Iyer, L.M., Liu, D.R., Aravind, L. & Rao, A. 2009. Conversion of 5-methylcytosine to 5-hydroxymethylcytosine in mammalian DNA by MLL partner TET1. *Science* **324**, 930–935.
- Terruzzi, I., Senesi, P., Montesano, A., La Torre, A., Alberti, G., Benedini, S., Caumo, A., Fermo, I. & Luzi, L. 2011. Genetic polymorphisms of the enzymes involved in DNA methylation and synthesis in elite athletes. *Physiol Genomics* **43**, 965–973.
- Tonkonogi, M. & Sahlin, K. 2002. Physical exercise and mitochondrial function in human skeletal muscle. *Exerc Sport Sci Rev* **30**, 129–137.
- Tost, J. 2008. Methods for the genome-wide and the gene-specific analysis of DNA methylation levels and patterns. In: J. Tost (ed.) *Epigenetics*, pp. 63–82. Caister Academic Press, Norfolk, UK.
- Ulrey, C.L., Liu, L., Andrews, L.G. & Tollefsbol, T.O. 2005. The impact of metabolism on DNA methylation. *Hum Mol Genet* **14** Spec No, R139–R147.
- Vincent, B., Windelinckx, A., Van Proeyen, K., Masschelein, E., Nielens, H., Ramaekers, M., Van Leemputte, M., Hespel, P. & Thomis, M. 2012. Alpha-actinin-3 deficiency does not significantly alter oxidative enzyme activity in fast human muscle fibres. *Acta Physiol* **204**, 555–561.
- Wanajo, A., Sasaki, A., Nagasaki, H., Shimada, S., Otsubo, T., Owaki, S., Shimizu, Y., Eishi, Y., Kojima, K., Nakajima, Y., Kawano, T., Yuasa, Y. & Akiyama, Y. 2008. Methylation of the calcium channel-related gene, CACNA2D3, is frequent and a poor prognostic factor in gastric cancer. *Gastroenterology* **135**, 580–590.
- White, A.J., Sandler, D.P., Bolick, S.C.E., Xu, Z., Taylor, J.A. & Deroo, L.A. 2013. Recreational and household physical activity at different time points and DNA global methylation. *Eur J Cancer* **49**, 2199–2206.
- You, J.S. & Jones, P.A. 2012. Cancer genetics and epigenetics: two sides of the same coin? *Cancer Cell* **22**, 9–20.
- Yu, W., Gius, D., Onyango, P., Muldoon-Jacobs, K., Karp, J., Feinberg, A.P. & Cui, H. 2008. Epigenetic silencing of tumour suppressor gene p15 by its antisense RNA. *Nature* **451**, 202–206.
- Yuasa, Y., Nagasaki, H., Akiyama, Y., Hashimoto, Y., Takizawa, T., Kojima, K., Kawano, T., Sugihara, K., Imai, K. & Nakachi, K. 2009. DNA methylation status is inversely correlated with green tea intake and physical activity in gastric cancer patients. *Int J Cancer* **124**, 2677–2682.
- Zarebska, A., Ahmetov, I.I., Sawczyn, S., Weiner, A.S., Kaczmarczyk, M., Ficek, K., Maciejewska-Karłowska, A., Sawczuk, M., Leonska-Duniec, A., Klocek, T., Voronina, E.N., Boyarskikh, U.A., Filipenko, M.L. & Cieszczyk, P. 2013. Association of the MTHFR 1298A>C (rs1801131) polymorphism with speed and strength sports in Russian and Polish athletes. *J Sports Sci* **32**, 375–382.
- Zeng, H., Irwin, M.L., Lu, L., Risch, H., Mayne, S., Mu, L., Deng, Q., Scarampi, L., Mitidieri, M., Katsaros, D. & Yu, H. 2011. Physical activity and breast cancer survival: an epigenetic link through reduced methylation of a tumor suppressor gene L3MBTL1. *Breast Cancer Res Treat* **133**, 2–3.
- Zhang, F.F., Cardarelli, R., Carroll, J., Fulda, K.G., Kaur, M., Gonzalez, K., Vishwanatha, J.K., Santella, R.M. & Morabia, A. 2011a. Significant differences in global genomic DNA methylation by gender and race/ethnicity in peripheral blood. *Epigenetics* **6**, 623–629.
- Zhang, F.F., Cardarelli, R., Carroll, J., Zhang, S., Fulda, K.G., Gonzalez, K., Vishwanatha, J.K., Morabia, A. & Santella, R.M. 2011b. Physical activity and global genomic DNA methylation in a cancer-free population. *Epigenetics* **6**, 293–299.
- Zhang, F.F., Santella, R.M., Wolff, M., Kappil, M.A., Markowitz, S.B. & Morabia, A. 2012. White blood cell global methylation and IL-6 promoter methylation in association with diet and lifestyle risk factors in a cancer-free population. *Epigenetics* **7**, 606–614.
- Zhang, B., Zhou, Y., Lin, N., Lowdon, R.F., Hong, C., Nagarajan, R.P., Cheng, J.B., Li, D., Stevens, M., Lee, H.J. et al. 2013. Functional DNA methylation differences between tissues, cell types, and across individuals discovered using the M&M algorithm. *Genome Res* **23**, 1522–1540.