Genetic polymorphisms of the enzymes involved in DNA methylation and synthesis in elite athletes

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Submitted 18 February 2010; accepted in final form 9 June 2011

Terruzzi I, Senesi P, Montesano A, La Torre A, Alberti G, Benedini S, Caumo A, Fermo I, Luzi L. Genetic polymorphisms of the enzymes involved in DNA methylation and synthesis in elite athletes. Physiol Genomics 43: 965–973, 2011. First published June 14, 2011; doi:10.1152/physiolgenomics.00040.2010.—Physical exercise induces adaptive changes leading to a muscle phenotype with enhanced performance. We first investigated whether genetic polymorphisms altering enzymes involved in DNA methylation, probably responsible of DNA methylation deficiency, are present in athletes’ DNA. We determined the polymorphic variants C667T/A1298C of 5,10-methylenetetrahydrofolate reductase (MTHFR), A2756G of methionine synthase (MTR), A66G of methionine synthase reductase (MTRR), G742A of betainehomocysteine methyltransferase (BHMT), and 68-bp ins of cystathionine β-synthase (CBS) genes in 77 athletes and 54 control subjects. The frequency of MTHFR (AC), MTR (AG), and MTRR (AG) heterozygous genotypes was found statistically different in the athletes compared with the control group (P = 0.0001, P = 0.018, and P = 0.0001), suggesting a reduced DNA methylating capacity. We therefore assessed whether DNA hypomethylation might increase the expression of myogenic proteins expressed during early (Myf-5 and MyoD), intermediate (Myf-6), and late-phase (MHC) of myogenesis in a cellular model of hypomethylation might increase the expression of muscle-specific protein expression in C2C12 myoblasts. Myogenic proteins are largely induced in hypomethylated cells [fold change (FC) = Myf-5: 1.21, 1.35; MyoD: 0.9, 1.47; Myf-6: 1.39, 1.66; MHC: 1.35, 3.10 in GMA, DNA, respectively; compared with the control groups (FC = Myf-5: 1.0, 1.38; MyoD: 1.0, 1.14; Myf-6: 1.0, 1.44; MHC: 1.0, 2.20 in GM, DM, respectively]).

Diameters and length of hypomethylated myotubes were greater then their respective controls. Our findings suggest that DNA hypomethylation due to lesser efficiency of poly- morphic MTHFR, MS, and MSR enzymes induces the activation of factors determining proliferation and differentiation of myoblasts promoting muscle growth and increase of muscle mass.

Gene expression is controlled by an adequate supply of methyl groups to the DNA. Specific mechanisms (Fig. 1) take part in controlling DNA methylation (35): the methionine synthase enzyme (MS) uses MS-bound cobalamin cofactor as intermediate methyl-carrier and N5-methyltetrahydrofolate as methyl donor, supplied by methylene-tetrahydrofolate reductase (MTHFR) enzyme. Methionine synthase reductase (MSR) plays a critical role in maintaining adequate levels of activated cobalamin, the cofactor for MS, which acts as an intermediate methyl carrier between methylenetetrahydrofolate and homocysteine, which is condensed with serine to form cysteine by the enzyme cystathionine β-synthase (CBS). The betaine-homocysteine methyltransferase (BHMT) enzyme uses betaine as a methyl donor to catalyze an alternative pathway of homocysteine remethylation, which, in humans, is mainly confined to the liver and the kidney (28). But the whole cycle performs other important functions: it controls gene expression by ensuring an adequate supply of methyl groups to the DNA; it also regulates nucleotides synthesis, and then the cell cycle, using methylene group attached to tetrahydrofolate to convert the uracil-type base found in RNA into the thymine-type base found in DNA.

The functional integrity of anyone of the enzymes regulating these pathways is important in maintaining adequate efficiency of this cycle: common variants in genes codifying regulatory enzymes of methylation cycle, caused by missense mutations, are characterized by a reduced enzyme activity (7, 39, 17, 15), probably responsible of both DNA methylation and synthesis modification.

The present work was therefore designed to investigate if the polymorphic variants of MTHFR C677T and A1298C, of CBS 844ins68, of methionine synthase (MTR) A2756G, of methionine synthase reductase (MTRR) A66G, of betaine:homocysteine methyltransferase (BHMT) G742A, and of CBS 68-bp ins, which disrupt the activity of the respective enzymes (7, 39, 17, 15), are present in the DNA of athletes. The study of the 76 elite athletes herein indicates this is the case. Since the presence of polymorphic variants in our cohort of athletes that may induce a reduction in DNA methylation, we also studied an in vitro model of DNA hypomethylation testing whether DNA hypomethylation would increase the expression of muscle-specific genes (Myf-5, MyoD, Myf-6, and MHC) crucial in the myogenic differentiation. In vitro experiments demonstrated that the induction of hypomethylation of DNA causes cell hypertrophy and hyperplasia. Taken together, our in vivo and in vitro studies indicate that elite athletes possess DNA polymorphisms of DNA methylation cycle enzymes that may...
predispose them to quicker DNA hypomethylation and, consequently, to a higher rate of DNA synthesis. We also showed that DNA hypomethylation in vitro determines increase in muscle cell size.

MATERIALS AND METHODS

Subjects. In a case control study, we examined the allelic frequencies and genotype distributions of restricted fragment length polymorphisms (RFLP) in the MTHFR, MTR, MTRR, BHMT, and CBS genes among elite athletes and sedentary subjects. Elite athlete is defined as an highly specialized athlete whose performances correspond to the best world results in his or her respective sports or discipline.

Of the 131 subjects recruited for the study, 54 (mean ± SD: BMI = 22.4 ± 1.8; yr = 32.3 ± 8.1) were studied as the sedentary control group and 77 subjects (mean ± SD: BMI = 21.0 ± 1.8; yr = 23.4 ± 5.0) represented the elite athlete group, studied independently from their training stage. All the subjects recruited for the study gave their informed written consent after being given an explanation of purposes, nature, and potential risks of the study.

In vitro experimental protocol. Myoblastic C2C12 cells were maintained in growth medium containing DMEM (Dulbecco’s modified eagle medium) supplemented with 20% (vol/vol) FBS (fetal bovine serum) up to 70% of confluence, and then (time 0) maintained in growth medium or differentiated in DMEM supplemented with 1% horse serum and antibiotics, both without (GM, DM) or with (GMA, DMA) 5-aza-2-deoxycytidine (AZA, 5 μM). At the end of the basal period (0), early (4 h), intermediate (24 h, 48 h), and late myogenesis (96 h), cells were lysed and the expression of specific myogenic genes (Myf-5, MyoD, Myf-6, and MHC) was detected by Western blotting. During late differentiation (72 and 96 h) morphology of MHC expressing cells was studied by immunofluorescence analysis. For each experimental condition the DAPI (4,6-diamidino-2-phenylindole)-stained nuclei per myotube and myotube length and diameter were determined and expressed as percentage increase ± SD.

Total homocysteine, vitamin B12, and vitamin B6 determination. Peripheral blood samples were obtained from the patients in the fasting state. Total plasma homocysteine levels were determined using an HPLC (high performance liquid chromatography) method based on the derivatization with SBD-F (ammonium-7-fluorobenzo-2-oxa-1,3-diazole-4-sulfonate) (6). Vitamin B12 was assayed by enzyme immunoassay using the Advia Centaur Immunoassay system (Bayer Health Care). Vitamin B6 (pyridoxal 5'-phosphate) was measured by applying a radioenzymatic assay (3).

Mutation detection. DNA from whole blood nuclear pellets, drawn after an overnight fast, was digested and quantified. For DNA extraction, all reagents were purchased from Sigma-Aldrich. PCR-based RFLP analysis was used for genotyping in this study. MTHFR (C677T) and MTHFR (A1298C) polymorphisms were analyzed as described (19, 36). Amplification products underwent digestion, respectively with Hinf I (MTHFR C677T) and Mbo II (MTHFR A1298C) (Promega, Madison, WI), and were then electrophoresed on a 3% methaphore agarose gel (Cambrex Bio Science Rockland).

The MTR (A2756G) and MTRR (A66G) polymorphisms were identified after amplification as described (42, 40). Restriction enzymes Hae III and Nde I (Promega) were used for RFLP analysis of amplification products. The primers and the conditions used for BHMT genotyping were the same described (38). The PCR product was digested with TaqI (Promega). For the 68-bp insertion variant of CBS gene, DNA was amplified with the primers described (19) and amplification products were observed on a 3% agarose gel.

Fig. 1. Simplified scheme of DNA methylation/synthesis cycle. Dihydrofolate (DHF), tetrahydrofolate (THF), methionine (MET), S-adenosylmethionine (SAM), S-adenosylhomocysteine (SAH), homocysteine (Hcy), 5,10-methylenetetrahydrofolate reductase (MTHFR), thymidylate synthase (TS) methionine synthase (MS), methionine synthase reductase (MSR), betaine:homocysteine methyltransferase (BHMT), cystathionine β-synthase (CBS), B6 vitamin (B6), and B12 vitamins (B12).
Electrophoretic techniques and immunoblotting analysis. C2C12 myotubes were homogenized as described (32). Aliquots of 30 μg supernatant proteins from the different samples were resolved by 8% SDS-PAGE. Electrophoresed proteins were transferred to nitrocellulose membrane (Protran, Whatman Schleicher & Schuell) as described (34). The membranes were incubated with Myf5 (C-20), MyoD (C-20), Myf6 (C-19), or MHC (H-300) antibodies and then incubated with horseradish peroxidase-conjugated anti-species-specific secondary antibodies. Immunoreactive bands were visualized by an enhanced chemiluminescence method (Amersham Pharmacia Biotech, Piscataway, NJ).

The membranes were stripped and reprobed with an antibody to α-tubulin (TU-02) to confirm equal protein loading per sample. All antibodies were purchased by Santa Cruz Biotechnology (Santa Cruz, CA). Quantitative measurement of immunoreactive bands was performed by densitometric analysis using the Scion image software (Scion, Frederick, MD).

Immunofluorescence analysis. For indirect immunofluorescence, cells were fixed in 4% paraformaldehyde, permeabilized with 0.2% Triton X-100, and blocked with PBS containing 1% bovine serum albumin. Cells were then immunostained with anti-MHC rhodamine conjugated (Santa Cruz Biotechnology) and nuclei revealed with DAPI staining. Cells were observed using fluorescence microscopy (Leica DM IRE2), and images of myotubes were captured using IM50 software (Leica Microsystems, Switzerland) for size comparison. Data were displayed and analyzed using Adobe Photoshop CS4.

Statistical analysis. Statistical differences between control and athlete genotype frequencies were calculated by using the Exact Test 1.0.0.1 for comparison. Data were displayed and analyzed using Adobe Photoshop CS4.

 statistical analysis. Statistical differences between control and athlete genotype frequencies were calculated by using the Exact Test 1.0.0.1 for Windows 95/98/NT 4.0/ME/2000/XP (http://www.exact-test.com/), generalization of Fisher’s Exact Test for 2 × 2 contingency tables. The exact P value was estimated by generation of 10,000,000 random samples. Expected genotype frequencies were obtained from the allele frequencies calculated for each genotype, under the assumption of Hardy-Weinberg (H-W) equilibrium by using the Exact Test, and were then compared with the observed frequencies by a Monte-Carlo simulation.

When the determined deviation between observed and expected numbers has a probability that is >5%, there is no statistical deviation from H-W equilibrium. Statistical significance calculated using Fisher’s Exact Test was interpreted as two-tailed P values of <0.05. Odds ratios (OR) and 95% confidence intervals (95% CI) were calculated to estimate the correlation between polymorphic genotypes and athletic phenotypes.

For immunoblotting and immunofluorescence analysis, statistical evaluations were performed by Student t-test using the SPSS 10.0 for Windows statistical package. Differences between groups were considered statistically significant if P ≤ 0.05.

RESULTS

Investigation of polymorphic variants in DNA methylation cycle genes. The mechanisms by which the DNA methylation cycle enzymes control DNA synthesis and methylation are summarized in Fig. 1. Genetic variants of these enzymes are responsible for a reduced efficiency in DNA synthesis and methylation. Table 1 shows that plasma levels of B12 (434.5 ± 131.4, 457.2 ± 146.5 pg/ml; P = 0.58) and vitamin B6 (32.4 ± 18.4, 36.4 ± 15.9 nmol/l; P = 0.20), well-known cofactors (25) crucial for the correct activity of MS and CBS enzymes, were normal in all subjects, and the values were not statistically different between the athlete group compared with the control group.

We compared the allele and genotype frequencies of the MTHFR (C677T and A1298C), MTR (A2756G), MTRR (A66G), BHMT (G742A), and CBS (68-bp ins) polymorphic genes in athletes and control subjects (Fig. 2). Both groups were divided according to their wild-type, heterozygous, and homozygous status relative to each of the six studied polymorphic sites (Table 2). A significantly increased frequency of only MTHFR (AC), MTR (AG), and MTRR (AG) heterozygous genotypes in the athletes’ group compared with the control group was observed (P = 0.0001, 0.018, and 0.0001). Comparison of the observed and expected frequencies demonstrated that there was a significant deviation in control subjects of (MTHFR) C677T and in athletes of (MTHFR) A1298C genotype frequencies from the H-W equilibrium (P = 0.03), probably explained by the small sample size used for this study and a random genetic selection for individuals with certain genotypes in the sampling process giving rise to a lower than expected number of subjects with the MTHFR homozygous genotypes.

Table 3 provides evidence for an increased predisposition of the athletic group for carrying a polymorphic genotype for the single MTHFR (AC), MTR (AG), or MTRR (AG and GG) gene compared with the sedentary genotypes at the same loci (P < 0.001, 0.02, <0.001, and 0.002, respectively) estimated at OR of 4.76 (95% CI: 2.2–10.3), 2.94 (95% CI: 1.2–6.9), 4.71 (95% CI: 2.1–10.3), and 17.14 (95% CI: 2.0–143.5), respectively.

Myogenic protein expression is increased by AZA-DNA hypomethylation. To simulate the effect of hypomethylation due to the less efficient polymorphic MTHFR, MS, and MSR enzymes, we caused DNA hypomethylation by AZA (5 μM) in murine C2C12 myoblastic cells and studied the regulation of muscle-specific genes expression during myoblast differentiation. C2C12 cells have been widely used as a model for the process of myogenesis. Under standard tissue culture conditions (GM), the cells proliferate as single cell myoblasts. When confluent cultures are transferred from GM to DM, the myoblasts exit cell cycle (Fig. 3A) and begin to elongate (early differentiation). Subsequently, confluent mononucleated myocytes begin to fuse forming multinucleate myotubes (intermediate differentiation), which become wider and longer over the next few days as additional myoblasts fuse (late differentiation).

Figure 3B shows that the myogenic factors progressively expressed during early phase (Myf-5 and MyoD) and intermediate phase (Myf-6) and the muscle-specific protein (MHC)
expressed during the late phase of myogenic differentiation are largely induced by DNA hypomethylation in GMA and DMA groups (FC/His Myf-5: 1.21, 1.35; MyoD: 0.9, 1.47; Myf-6: 1.39, 1.66; MHC: 1.35, 3.10, respectively) compared with the GM and DM groups (FC/His Myf-5: 1.0, 1.38; MyoD: 1.0, 1.14; Myf-6: 1.0, 1.44; MHC: 1.0, 2.20, respectively). In addition, the expression of MHC gene results positively regulated by hypomethylation with respect to the unfhypomethylated differentiated muscle cells. Overall, our results demonstrate that MHC gene expressed in muscle tissues is transcriptionally regulated by DNA hypomethylation.

The hypertrophic response of myofibers is induced by AZA-DNA hypomethylation.

To validate the effect of DNA hypomethylation on myoblast differentiation and to investigate whether that effect could improve the recruitment of myoblasts into myotubes, or myotube hypertrophy, C2C12 cells were cultured in GM...
or DM in the presence or absence of AZA for 72 or 96 h, respectively.

Images of MHC-positive myotubes detected by immunofluorescence (Fig. 4, A and a) showed that the morphology of cells that were hypomethylated by AZA in growth (GMA) or differentiation medium (DMA) for 3 and 4 days was severely altered relative to their respective control GM and DM.

The process by which cells with star-shaped morphology typical of the early stages of differentiation were substituted with elongated spindle-shaped cells, which aligned close to each other in the later stages, was slower in GM and DM (96 h) than in GMA and DMA (72 h). In C2C12 cells cultured in DM or GM in the absence of AZA, maximal myoblast fusion and myotube dimension required longer times than in AZA-stimulated cells. After 72 h of treatment, the length and diameter percentage increase (Fig. 4, B and C) of the myotubes cultured in GMA was 34.9 and 57.2% with respect to GM, whereas that of myotubes in DMA was 25.0 and 23.7% with respect to DM. At 96 h of differentiation, the length percentage increase of the myotubes (Fig. 4b) treated with AZA is even greater than their respective controls (GMA = 18.7% with respect to GM and DMA = 17.7% with respect to DM). The diameter percentage increase of the cells differentiated without AZA (DM) seems to stop when they reach the same GM myotube size (DM = 3.7% with respect to GM), while the diameter percentage increase of the AZA-stimulated cells is still growing (GMA = 75.8%, DMA = 79.6% with respect to GM and DM) as shown in Fig. 4c.

**DISCUSSION**

Our in vivo studies in a cohort of elite athletes demonstrate the presence of DNA polymorphisms of enzymes involved in the homocysteine cycle leading to reduced DNA methylation. Therefore, we set up for in vitro experiments where DNA hypomethylation was induced in murine myoblasts demonstrating an higher rate of activation and differentiation in muscle fibers, leading to muscle hypertrophy and hyperplasia. Taken together, in vivo and in vitro data indicate that elite athletes have a genetic predisposition to DNA hypomethylation.

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Table 3. *Ratio of odds of expressing the polymorphic genotypes in control group to the odds of expressing the same polymorphisms in athlete group*

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>OR (control/athletes)</th>
<th>95% CI</th>
<th>P</th>
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<tbody>
<tr>
<td>MTHFR (C677T)</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>CC</td>
<td>0.73</td>
<td>0.3–1.6</td>
<td>0.44</td>
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<tr>
<td>CT</td>
<td>0.78</td>
<td>0.2–2.9</td>
<td>0.74b</td>
</tr>
<tr>
<td>TT</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>MTHFR (A1298C)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AA</td>
<td>4.76</td>
<td>2.2–10.3</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>AC</td>
<td>1.62</td>
<td>0.2–12.4</td>
<td>0.64b</td>
</tr>
<tr>
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<td></td>
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<tr>
<td>MTR (A2756G)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AA</td>
<td>2.94</td>
<td>1.2–6.9</td>
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<tr>
<td>AG</td>
<td>0.91</td>
<td>0.1–15.0</td>
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<td>MTRR (A66G)</td>
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<tr>
<td>AA</td>
<td>4.71</td>
<td>2.1–10.3</td>
<td>&lt;0.001</td>
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<tr>
<td>AG</td>
<td>17.14</td>
<td>2.0–143.5</td>
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<td>GG</td>
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<tr>
<td>BHMT (G742A)</td>
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<tr>
<td>GA</td>
<td>0.94</td>
<td>0.4–2.0</td>
<td>0.87</td>
</tr>
<tr>
<td>AA</td>
<td>2.30</td>
<td>0.4–12.0</td>
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<tr>
<td>wt homo</td>
<td>1.48</td>
<td>0.4–6.2</td>
<td>0.73b</td>
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OR, odds ratio; CI, confidence interval. bFisher’s exact test (2 sided) computed for 2x2 contingency table.
Fig. 4. Effects of AZA on myoblast differentiation. MHC in myotubes of control (GM, DM) and treated with AZA (GMA, DMA) was detected by immunofluorescence 72 h (A) and 96 h (a) after differentiation. The diameters of MHC-positive myotubes differentiated for 72 h (B) and 96 h (b) were measured and the average, expressed in cm, were graphically represented. The length of MHC-positive myotubes differentiated for 72 h (C) and 96 h (c) were measured and the average, expressed in cm, were graphically represented. The data of 3 independent experiments are expressed as means \pm SD.
and synthesis that we demonstrated to be some of the factors leading to myogenic differentiation stimulation and muscle mass increase (Fig. 5).

The primary mechanisms involved in muscle mass increase are the proliferative activation (22, 23) and myogenic differentiation of mononuclear satellite cells that fuse with the enlarging myofiber, as well as an increased protein synthesis (10, 14, 30), although some evidence is present that hyperplasia of existing muscle fibers also plays a role in muscle enlargement along with hypertrophy alone (1).

Several factors besides DNA hypomethylation induce muscle hypertrophy. Skeletal muscle-specific growth factors and hormones modulate satellite cell activity during muscle growth and development; however, a single bout of voluntary high-intensity exercise was shown to increase the number of satellite cells, and repeated bouts of exercise are sufficient for the satellite cell to undergo terminal differentiation (5), indicating in physical exercise a major stimulus in muscle hypertrophy. Therefore, in elite athletes, two major stimuli inducing muscle hypertrophy are present, one hereditary (DNA hypomethylation due to polymorphic variants of enzymes of the DNA methylation cycle) and one environmental (high intensity and frequency of physical exercise training).

Studies in human bladder smooth muscle cells (16) have confirmed that a cyclic stretch-relaxation signal is able to increase the rate of protein synthesis and accelerate entry into the S phase, when DNA synthesis or replication occurs, inducing both hypertrophic and hyperplastic responses.

An adaptive response to exercise training is the increment of active muscle mass achieved by an increase in the volume of individual myofibers (11). To sustain hypertrophic enlargement, myofibers need the insertion of new nuclei. Muscle satellite cell proliferation, differentiation, and fusion with the existing myofibers are known to be responsible for postnatal

Fig. 5. Proposed skeletal muscle mechanism derived from the study data. Our results suggest that in athletes the increased speed of entry into S phase, and then DNA synthesis, stimulated by physical exercise, should be facilitated by the reduced enzymatic activity due to the polymorphic MTHFR gene, which increases 5-10-methylenetetrahydrofolate availability. A larger amount of substrate becomes thus available for the increased speed of entry into S phase and then DNA synthesis, stimulated by physical exercise facilitating hypertrophic responses. Likewise, as a consequence of the less efficient MS and MSR enzymes due to genetic variants as MTR (AG) and MTRR (AG) heterozygous genotypes, cellular 5-methyltetrahydrofolate is depleted, inducing DNA hypomethylation and a consequent increase of muscle-specific genes (Myf-5, MyoD, Myf-6 and MHC) expression, crucial in the myogenic differentiation. The data we obtained support our hypothesis of a functional role for the studied genetic variants to determine a role in athletic performance and identify polymorphisms of these genes as mediators of the myogenic and hypertrophic effects exerted by physical exercise on skeletal muscles.
muscle hypertrophy, by supplying more DNA to the individual fiber (4). One of the main physiological functions of methylation cycle can be classified as DNA synthesis by the activity of MTHFR enzyme responsible for transferring the methyl group from 5-10-methylenetetrahydrofolate to uracil, converting it to thymine used for DNA synthesis and repair (26). The reduced MTHFR enzyme activity (7), due to the C677T gene polymorphism, engenders an accumulation of 5-10-methylenetetrahydrofolate, increasing the availability of methyl groups.

Our data suggest that in athletes the increased speed of entry into S phase, and then DNA synthesis, stimulated by physical exercise, should be facilitated by the presence of MTHFR polymorphisms, which increase the 5-10-methylenetetrahydrofolate availability.

Moreover, DNA stability is affected also through another pathway (see Fig. 1) regulated by MS and MSR enzyme activity. 5-Methyltetrahydrofolate also serves as methyl donor in the methylation of specific cytosines in DNA, an epigenetic mechanism to exert transcriptional control and regulate gene transcription.

While in vitro models are routinely used to try to elucidate signaling pathways, the genetic factors influencing muscle response to exercise training are more difficult to dissect out, although correlation studies of polymorphic variation associated with muscle size and strength were performed (33, 24), and several papers have been published on this topic.

The role of DNA methylation as a locking mechanism for an important event, such as tissue-specific gene expression during development, is well established (8, 27, 20). In particular, several studies of single muscle genes (2, 12, 18, 37) have demonstrated a role of hypomethylation in the induction of muscle differentiation (21, 29, 31). Of the several tissue-specific transcription factors, a family of transcriptional regulators (MyoD, Myf-5, and Myf-6) is known to be closely involved in the commitment to myogenic fate and in the induction of muscle-specific genes expression (MHC).

It might be expected that, in athletes, as a consequence of reduced MTHFR, MS, and MSR enzyme activity due to genetic variants [namely MTHFR (AC), MTR (AG), and MTRR (AG) heterozygous genotypes], cellular 5-methyltetrahydrofolate is depleted, inducing DNA hypomethylation and a consequent increase of muscle-specific genes expression. To verify this hypothesis, we utilized a murine cell line model to study the effects of DNA hypomethylation by AZA (5 μM), a DNA methyltransferase inhibitor, on the regulation of muscle-specific genes expression, myoblast differentiation, and hypertrophy, using the mouse myoblast C2C12 cell line model system. This immortalized cell line derived from satellite cells has routinely been used as a model for skeletal muscle development and skeletal muscle differentiation. In these cells, DNA hypomethylation increased the expression of the myogenic regulatory factors Myf-5 and MyoD, required for myogenic determination, and Myf-6, which has a role in terminal differentiation inducing an increment in MHC muscle-specific gene expression, confirming that the mononucleated cells finally become muscle cells. The greater capability of hypomethylated cells, not only to differentiate earlier in mature muscle cell, but also to support an hypertrophy process that leads treated cells to reach length and diameter size considerably higher than unhypomethylated cells is confirmed by the immunofluorescence images.

Elite athletes have normally an high insulin sensitivity. Although several substrates and cofactors involved in the methylation cycle are shown to modulate insulin action, the level of homocysteine, involved in this metabolic pathway, is important for the interpretation of the present findings. Li et al. (13) showed that high homocysteine concentration impairs insulin sensitivity via a proinflammatory action and a resistance-mediated effect. In our athletes bearing the polymorphic variants, the methylation of homocysteine to methionine is reduced so that it is very likely that the all cycle flux is down regulated. In contrast, CBS function was found to be normal in our athletes, explaining the normal homocysteine levels. More directly related to insulin sensitivity are probably polymorphisms of the respiratory chain enzymes, not under study in this experimental setting.

In conclusion, the significant increase of MTHFR A1298C, MTR A2756G, and MTRR A66G polymorphic variant frequency, in athletes with respect to controls, leads us to speculate that these polymorphisms represent a genetic factor that determines athletic performance in association with other environmental factors. Significant progress has been made in recent years to understand the mechanisms that induce both skeletal muscle differentiation and hypertrophy, but our data provide the first evidence of a potential functional role for the genetic variants studied herein to determine a role in athletic performance. Therefore, the search for polymorphisms of these genes as mediators of the myogenic and hypertrophic effects exerted by exercise on skeletal muscles may become a routine genetic test in sport medicine.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

REFERENCES

ATHLETES AND METHYL CYCLE ENZYMES POLYMORPHISMS


