Defective Mitochondrial Function In Vivo in Skeletal Muscle in Adults with Down’s Syndrome: A $^{31}$P-MRS Study

Alexander C. Phillips$^1$, Alison Sleigh$^2$, Catherine J. McAllister$^1$, Soren Brage$^3$, T. Adrian Carpenter$^2$, Graham J. Kemp$^4$, Anthony J. Holland$^1$

1 Department of Psychiatry, University of Cambridge, Cambridge, United Kingdom, 2 Wolfson Brain Imaging Centre, University of Cambridge, Cambridge, United Kingdom, 3 MRC Epidemiology Unit, Institute of Metabolic Science, University of Cambridge, Cambridge, United Kingdom, 4 Department of Musculoskeletal Biology, University of Liverpool, Liverpool, United Kingdom

Abstract

Down’s syndrome (DS) is a developmental disorder associated with intellectual disability (ID). We have previously shown that people with DS engage in very low levels of exercise compared to people with ID not due to DS. Many aspects of the DS phenotype, such as dementia, low activity levels and poor muscle tone, are shared with disorders of mitochondrial origin, and mitochondrial dysfunction has been demonstrated in cultured DS tissue. We undertook a phosphorus magnetic resonance spectroscopy ($^{31}$P-MRS) study in the quadriceps muscle of 14 people with DS and 11 non-DS ID controls to investigate the post-exercise resynthesis kinetics of phosphocreatine (PCr), which relies on mitochondrial respiratory function and yields a measure of muscle mitochondrial function in vivo. We found that the PCr recovery rate constant was significantly decreased in adults with DS compared to non-DS ID controls ($1.7 \pm 0.1 \text{ min}^{-1}$ vs $2.1 \pm 0.1 \text{ min}^{-1}$ respectively) who were matched for physical activity levels, indicating that muscle mitochondrial function in vivo is impaired in DS. This is the first study to investigate mitochondrial function in vivo in DS using $^{31}$P-MRS. Our study is consistent with previous in vitro studies, supporting a theory of a global mitochondrial defect in DS.

Methods

Ethics Statement

Ethical approval for the study was granted by the Cambridgeshire 3 Research Ethics Committee and all studies were conducted in accordance with the principles of the Declaration of Helsinki.
Only participants judged to have the capacity to consent in accordance with the Mental Capacity Act (2005) were recruited. Written consent was obtained from all participants.

Recruitment, Screening and Familiarisation

Participants with ID not due to DS were deliberately chosen as controls for the DS group in an attempt to match for level of intellectual disability, environmental factors and fitness levels. Participants were recruited from a database of participants in a previous study [3] and were initially selected to match as far as possible age, gender, BMI and physical activity levels between groups. We scanned 29 individuals, but after detailed consideration when matching activity levels of the two groups, selected for analysis 14 DS and 11 ID participants. Inclusion criteria were: mild to moderate ID; known to ID services; aged ≥12 y; able to walk unaided; and able to understand protocol instructions. Exclusion criteria were: smoking; autism spectrum disorder; diabetes mellitus, cardiovascular disease; medication known to affect energy metabolism; and standard magnet contraindications. Prior to inclusion, participants underwent a preliminary screening at their home residence to determine whether they would be able to understand and complete the study protocol, and to assess their capacity to consent. Two weeks prior to the experimental measurements participants were shown a video and underwent at least two familiarisation sessions at their home residence to ensure they were comfortable with the in-scanner exercise protocol. All participants underwent at least four separate practice trials.

Measurements of Height, Weight, and Body Fat

Weight was measured to the nearest 0.1 kg with the participant dressed in lightweight clothing, and without shoes, using a calibrated electronic scale (Seca 813, Seca Ltd, UK). Height was measured by the stretch stature method to the nearest 0.5 cm using a portable stadiometer (Seca Leicester Height Measure, Invicta Plastics, UK). Percentage body fat was assessed by bioelectrical impedance using a TBF-521 analyser (Tanita Corp., Japan) in accordance with the manufacturer’s instructions.

Measurement of Leg Strength

Two weeks prior to the experimental measurements isometric leg strength was measured at the participants’ home residence using a portable back and leg dynamometer (Takei, model T.K.K. 5162, Japan) according to a protocol [10] which has been validated in both adolescents and adults [10,11].

Assessment of Total Physical Activity

Habitual physical activity was assessed using an Actigraph GT1M accelerometer (Actigraph LLC, Pensacola, FL, USA) for seven consecutive days. Accelerometers have been used previously to assess physical activity in a number of studies in DS [12–14]. The total physical activity measure was used as a measure of habitual physical activity in these participants and not used to make inferences on energy expenditure [14]. These participants formed part of a previous report on activity in these cohorts and the methodology and analysis were as previously described in that study [5].

$^{31}$P-MRS Measurement of Post-exercise PCr Recovery Kinetics

$^{31}$P-MRS data were acquired on a Siemens MAGNETOM 3T Verio scanner. Participants were asked to arrive having refrained from strenuous exercise for at least 24 h and having abstained from alcohol, caffeine, and food for the preceding 24 h, 6 h, and 2 h respectively. The volunteers were placed supine and a 9 cm diameter surface coil attached to the right quadriceps (1/3 distal). An MR-compatible weight was attached to the right ankle that was proportional to their previously-determined leg strength, such that the PCr concentration after exercise was targeted to fall by 20–30% of basal levels, thereby avoiding any significant lowering of pH which is known to complicate interpretation of PCr recovery kinetics. The exercise paradigm consisted of 1 min rest, 1 min knee extensions (0.5 Hz), then 4 min rest. This was then repeated to permit two measurements of post-exercise PCr recovery, results from the analysis of which were averaged. Spectra were acquired with a TR of 2s and as previously described [15]. The PCr recovery rate constant, k, was found using a two parameter monoeXponential fit to the equation: \[PCr(t) = PCr_{initial} + (PCr_{end} - PCr_{initial}) (1 - \exp(-kt))\], where t is the time from the start of recovery, PCr_{initial} and PCr_{end} are the PCr content at the initial and end of recovery phases respectively. MATLAB software (The MathWorks, Natick, MA, USA) was used to calculate PCr_{end} from the mean end-recovery PCr values and then to obtain k and PCr_{initial} by the Levenberg Marquardt fitting algorithm. All spectra were analysed in jMRUI [16] and fitted using the AMARES [17] algorithm with prior knowledge. The intracellular pH was determined from the chemical shift of inorganic phosphate relative to PCr. A fully relaxed spectrum with 8 averages was also obtained for the calculation of metabolite ratios and concentrations. The conventional assumption was made that the intracellular concentration of ATP was 0.2 mM, and the free concentration of adenosine diphosphate (ADP) was calculated using established methods [18] with the conventional assumption (in the absence of any reported data in DS) of total creatine = 42.5 mM. The signal-to-noise ratio (SNR) of the PCr recovery sequence was defined as the mean signal/SD of the recovered PCr values (20 points, TR = 2s). High subcutaneous adipose tissue thicknesses can result in a low SNR and uncertainty in the accuracy of the PCr recovery rate constant, and hence a cut off value of SNR <35 was chosen for exclusion of k values.

Statistics

Statistical analysis was performed in IBM SPSS Statistics 21 (IBM Inc., Armonk, NY, USA), with significance set at $p<0.05$. A two-tailed independent samples t-test was performed to detect significant differences between groups.

Results

Participants with ID not due to DS and participants with DS were matched for age, gender, BMI and physical activity levels, and were found to have similar percentage body fat (Table 1). However, the DS were significantly shorter than the ID controls ($p=0.001$). No significant correlation of total physical activity with age was found in either group, or altogether.

Four participants with DS were eliminated from the calculation of k due to not meeting the SNR requirements described in the Methods section, and hence the $^{31}$P-MRS and accelerometry results in Table 2 represent the DS group with $n = 10$. Post-exercise PCr recovery was significantly slowed in the DS group compared with ID ($p=0.039$, Figure 1). Neither the resting nor end-exercise intracellular pH (pHi) differed significantly between those with DS and those with ID without DS (Table 2), and end-exercise pHi was not significantly lower than resting pHi in either group. No differences in resting metabolite concentrations nor their ratios were found between the groups (Table 2). There was
no significant correlation between total physical activity and the PCr recovery rate constant, k in either group, or altogether.

Discussion

We found significantly slower post-exercise PCr recovery in skeletal muscle of the participants with DS compared with the non-DS ID control participants. Post-exercise PCr recovery, conveniently measured as the recovery rate constant, which was 16% lower in the DS participants, is a measure of mitochondrial function in vivo which integrates the contributions of muscle mitochondrial numbers, the mitochondrial content of enzymes, transporters and respiratory chain complexes, the activity of relevant cytosolic enzymes, and the whole-body cardiorespiratory physiology affecting substrate and oxygen supply to active muscle [19]. In exercise of the kind used here the exercising muscle mass is very small (compared e.g. to bicycle ergometry [20]), and cardiovascular limitations to this measure are normally negligible. Our participants with DS had no clinical evidence of cardiovas-

cular or pulmonary disease, and therefore no reason to suppose that the defect we identified lies ‘upstream’ of the mitochondrion. The likeliest explanation is either a lower mitochondrial content, or a defect in intrinsic mitochondrial metabolism.

There are various lines of in vitro evidence to support, or at least parallel, our findings of mitochondrial dysfunction in vivo in DS. Previous in vitro studies in DS have shown mtDNA mutations or mitochondrial dysfunction in brain [6,21–23] and dysregulation of genes associated with mitochondrial function in foetal heart tissue [7]. More specifically lower mRNA levels of complex I and decreased protein levels in complex I subunits [21] as well as decreased levels of complex V [24] have been identified in the brains of people with DS. Levels of mitochondrial enzymes in platelets, including cytochrome oxidase (COX), have been shown to be significantly lower in people with DS compared with controls [8]. There is also evidence of compensation for mitochondrial inefficiency, including a 25% increase in mitochondrial mass of fibroblasts in people with DS [25]. A recent study by Helguera et al. [26] has shown that mitochondria move more slowly in DS neurons compared with controls, although there were higher numbers of moving mitochondria in DS at any given time that may be a compensatory mechanism. Collectively the results from these studies suggest a systemic long standing dysfunction of mitochondria in DS. To our knowledge, there have been no such studies in DS investigating skeletal muscle, which for practical reasons is the only tissue in which mitochondrial function in vivo (i.e. the capacity for mitochondrial ATP synthesis) can be quantified non-invasively [19]. The results of our in vivo study in skeletal muscle support this theory of a global mitochondrial defect.

We have previously shown that people with DS have highly sedentary lifestyles compared to their non-DS ID peers [5]. Similarly low levels of habitual physical activity have recently been reported in people with mitochondrial disease [27]. In addition, the authors of that study found a high prevalence of obesity, and a moderate negative correlation between steps walked per day and disease severity. We suggest that low levels of activity, the reduced

Table 1. Characteristics of the participants with Down’s syndrome (DS) and those with a non Down’s syndrome related intellectual disability (ID).

| Category          | Subcategory                  | DS (n = 14) | ID (n = 11) | p-value |
|-------------------|------------------------------|-------------|-------------|---------|
|                   | Age (yrs)                    | 32.7 (2.2)  | 29.8 (2.7)  | 0.410   |
|                   | Gender                       | M, 6 F      | M, 6 F      | 0.580   |
|                   | Weight (kg)                  | 72.2 (3.2)  | 80.0 (4.9)  | 0.175   |
|                   | Height (m)                   | 1.55 (0.03) | 1.72 (0.04) | 0.001   |
|                   | BMI (kg/m²)                  | 30.4 (1.7)  | 26.9 (1.1)  | 0.100   |
|                   | Percentage body fat (%)      | 27.5 (2.7)  | 27.5 (3.1)  | 0.991   |

Data presented are mean (SEM).

Table 2. Accelerometry and 31P-MRS measurements for the participants with Down’s syndrome (DS) and those with a non Down’s syndrome related intellectual disability (ID).

| Category       | Subcategory                  | DS (n = 10) | ID (n = 11) | p-value |
|----------------|------------------------------|-------------|-------------|---------|
| Accelerometry  | Total physical activity (counts/min) | 542.5 (25.3) | 611.6 (35.1) | 0.133   |
| 31P-MRS        | Exercise weight (kg)         | 2.03 (0.10) | 2.77 (0.29) | 0.031   |
|                 | k (min⁻¹)                    | 1.72 (0.10) | 2.06 (0.12) | 0.039   |
|                 | Resting Pi:PCr               | 0.086 (0.005) | 0.096 (0.006) | 0.216   |
|                 | Resting PDE:PCr              | 0.123 (0.011) | 0.115 (0.007) | 0.567   |
|                 | Resting Pi:PiPDE             | 0.73 (0.06) | 0.86 (0.07) | 0.213   |
|                 | Resting [Pi] (mM)            | 2.85 (0.16) | 2.96 (0.17) | 0.633   |
|                 | Resting [PDE] (mM)           | 4.07 (0.35) | 3.58 (0.22) | 0.244   |
|                 | Resting [PCr] (mM)           | 33.4 (0.7)  | 31.2 (0.9)  | 0.073   |
|                 | End exercise [PCr] (mM)      | 26.5 (1.5)  | 24.3 (0.9)  | 0.211   |
|                 | Resting [ADP] (µM)           | 14.7 (1.8)  | 20.2 (2.3)  | 0.078   |
|                 | End exercise [ADP] (µM)      | 36.8 (4.9)  | 45.2 (4.6)  | 0.227   |
|                 | Resting pH [6]               | 7.017 (0.015) | 7.036 (0.007) | 0.281   |
|                 | End exercise pH [6]          | 7.061 (0.010) | 7.068 (0.009) | 0.648   |

Data presented are mean (SEM). k, PCr recovery rate constant; [Pi], concentration of inorganic phosphate; [PCr], concentration of phosphocreatine; [PDE], concentration of phosphodiester; [ADP], concentration of adenosine diphosphate; pH, intracellular pH. doi:10.1371/journal.pone.0084031.t001
capacity for exercise [4] and high rates of obesity [28] in DS could be accounted for in part by reduced mitochondrial ability of skeletal muscle to generate ATP.

However, any cross-sectional study relating habitual activity to muscle energetics (in this case, muscle mitochondrial capacity) must address the issue of causal direction; whilst mitochondrial dysfunction \textit{in vivo} can certainly contribute to reduced habitual activity, it is also possible that reduced activity may lead to impaired muscle mitochondrial function. In the present study we have tried to address this by matching the DS and non-DS ID groups for physical activity. The data does not exist to say how much a ‘primary’ reduction in habitual activity would be necessary to yield the 16% defect we observed in mitochondrial function \textit{in vivo} in DS compared to non-DS ID, nor conversely how much a primary mitochondrial defect (in mitochondrial numbers or intrinsic mitochondrial activity) of this size would be expected, on its own, to limit activity. We do know that the ‘slope’ of the relationship between these two measures is steep, in the sense that symptomatic primary mitochondrial myopathy is associated with on average a 40–50% defect in skeletal muscle mitochondrial capacity as measured by $^{31}$P-MRS [29]. The defect we have identified here is therefore likely to be functionally significant, even if we cannot be sure whether it is cause or effect (or both).

Precocious ageing and Alzheimer’s disease (AD) are strikingly common in DS, with almost 100% of people with DS eventually developing the neuropathology of AD [30], which is likely to be linked to the overexpression of the amyloid precursor protein gene (\textit{APP}) resulting in a lifelong excess of beta-amyloid (A\textbeta). The role of mitochondrial dysfunction in neurodegenerative diseases is well-established [31]. A\textbeta has been shown to be imported into mitochondria where it inhibits function [32,33], and data indicate that mitochondrial dysfunction contributes to the development of DSAD [34]. A\textbeta deposits have been seen in non-brain tissue of people with AD, DS and in normal ageing [35]. Further, transfer of the \textit{bAPP} gene into cultured normal human muscle leads to decreased mitochondrial enzyme activity and structural abnormalities [36]. This demonstrates that mitochondrial interaction with A\textbeta is not limited to brain, and suggests, further, that mitochondrial dysfunction quantified in muscle \textit{in vivo}, as in the current study, may provide insight into A\textbeta and mitochondria in...
Mitochondrial dysfunction and mutations of mtDNA are a major hallmark of human ageing [37], suggesting that mitochondria may underlie the precarious biological ageing that is characteristic of DS. In the current study, there was no significant correlation with age in the DS group (p = 0.165, data not shown) although the eldest person in the group was only 44 y.

Low activity levels in DS are associated with reduced life expectancy, irrespective of heart disease [38]. Increasing mobility of people with DS should therefore improve well-being as well as mortality. Furthermore, high levels of exercise in people over 65 y in the general population has been shown to be protective against cognitive decline [39], adding further support to the notion that reduced habitual activity in DS may impact on the rate and severity of dementia, although mitochondrial dysfunction may underlie both these aspects of the phenotype. Understanding reasons for reduced habitual activity in DS, including the role of mitochondrial dysfunction and accumulation of mtDNA mutations, may be key to a number of health issues associated with the syndrome.

**Acknowledgments**

We are grateful to Louise McGrath for help in acquiring the data. We thank all the participants who took part in the study and the support workers and parents for supporting the participants during the study. We acknowledge Dr Dan Gordon, Anglia Ruskin University, for helpful discussions and the loan of the portable back and leg dynamometer.

**Author Contributions**

Conceived and designed the experiments: ACP AJH. Performed the experiments: ACP. Analyzed the data: AS GJK. Contributed reagents/materials/analysis tools: AS SB TAG. Wrote the paper: ACP AS CJM GJK.

**References**

1. Frid C, Deott P, Lundell B, Rasmussen F, Anneren G (1999) Mortality in Down’s syndrome in relation to congenital malformations. J Intellect Disabil Res 43 (Pt 3): 234–241.

2. Stoll C, Amlnik B, Dott B, Roth MP (1998) Study of Down syndrome in 230,942 consecutive births. Ann Genet 41: 44–51.

3. Rosasi NJ, Patterson D (2005) Down’s syndrome. Lancet 366: 1291–1298.

4. Mendosou G, Pereira FD, Fernhall B (2010) Reduced exercise capacity in persons with Down syndrome: cause, effect, and management. Ther Clin Risk Manag 6: 601–610.

5. Phillips AC, Holland AJ (2011) Assessment of objectively measured physical activity levels in individuals with intellectual disabilities with and without Down’s syndrome. PLoS One 6: e28618.

6. Coskun PE, Wyrembalk J, Derberhe O, Melloniam G, Doran E, et al. (2010) Systemic mitochondrial dysfunction and the etiology of Alzheimer’s disease and down syndrome dementia. J Alzheimers Dis 20 Suppl 2: S293–310.

7. Conti A, Fabbrini F, D’Agostino P, Negri R, Greco D, et al. (2007) Altered expression of mitochondrial and extracellular matrix genes in the heart of human fetuses with chromosome 21 trisomy. BMC Genomics 8: 266.

8. Paton J, Taw S, Bave U, Anneren G, Oerland L (1994) Mitochondrial enzyme deficiencies in Down’s syndrome. J Neurol Neurosurg Psychiatry 57: 171–181.

9. Larson-Meyer DE, Newcomer BR, Hunter GR, McLean JP, Hetherington HP, et al. (2000) Effect of weight reduction, obesity predisposition, and aerobic fitness on skeletal muscle mitochondrial function. Am J Physiol Endocrinol Metab 278: E153–161.

10. Coldwells A, Atkinson G, Reilly T (1994) Sources of variation in back and leg dynamometry. Ergonomics 37: 79–86.

11. Jawis MN, Singh R, Singh HJ, Yassin MN (2005) Anthropometric and clinical characteristics of people with Down syndrome. J Intellect Disabil Res 49 (Pt 3): 234–241.

12. Whitt-Glover MC, O’Neill KL, Stettler N (2006) Physical activity patterns in persons with Down syndrome: cause, effect, and management. Ther Clin Risk Manag 6: 601–610.

13. Shields N, Dodd KJ, Abblitt C (2009) Do children with Down syndrome have increased physical activity than non-handicapped children? J Physiol 590: 2621–2635.

14. Agiovlasitis S, Motl RW, Fahs CA, Ranadive SM, Yan H, et al. (2011) Impairment of F1F0-ATPase, adenine nucleotide translocator and adenylate kinase cause mitochondrial energy deficit in human skin fibroblasts with chromosome 21 trisomy. Biochem J 439: 299–310.

15. Sleigh A, Raymond-Barker P, Thackray K, Porter D, Hatunic M, et al. (2011) Mitochondrial dysfunction in patients with primary congenital insulin resistance. J Clin Invest 121: 2457–2461.

16. Taylor DJ, Kemp GJ, Radda GK (1994) Bioenergetics of skeletal muscle in Down’s syndrome. Arch Neurol 51: 849–853.

17. Joachim CL, Mori H, Selkoe DJ (1989) Amyloid beta-protein deposition in synaptic terminals and the amyloid beta-protein gene using adenovirus vector causes mitochondrial dysfunction in vivo. J Biol Chem 264: 18504–18510.

18. Lai F, Williams RS (1989) A prospective study of Alzheimer disease in Down syndrome. Arch Neurol 46: 849–853.

19. Helguera P, Scigle J, Rodriguez J, Hanna M, Helguera G, et al. (2013) Adaptive downregulation of mitochondrial function in down syndrome. Cell Metab 17: 132–140.

20. Mortensen SP, Damsgaard R, Dawsen EA, Secher NH, Gonzalez-Alonso J (2008) Restrictions in systemic and locomotor skeletal muscle perfusion, oxygen supply and VO2 during high-intensity whole-body exercise in humans. J Physiol 590: 2621–2635.

21. Kim SH, Fountoulakakis M, Dierssen M, Lubec G (2001) Decreased protein levels of complex I and complex V beta chain in brains from patients with Alzheimer’s disease. J Neurotransm 108: 109–116.

22. Busciglio J, Pelsman A, Wong C, Pagios G, Yuan M, et al. (2002) Altered metabolism of the amyloid beta precursor protein is associated with mitochondrial dysfunction in Down’s syndrome. Neurosurg Focus 13: 1–15.

23. Kim SH, Vilkonsky R, Cairns N, Lubec G (2000) Decreased levels of complex III core protein 1 and complex V beta chain in brains from patients with Alzheimer’s disease and Down syndrome. Cell Mol Life Sci 57: 1810–1816.

24. Lee SH, Lee S, Jun HS, Jeong HJ, Cha WT, et al. (2003) Expression of the mitochondrial ATPase gene and Tiam in Down syndrome. Mol Cells 13: 101–105.

25. Valenti D, Tullio A, Caratuzzo MF, Mefarina RS, Scarpellini P, et al. (2010) Systemic mitochondrial dysfunction and the etiology of Alzheimer’s disease and down syndrome dementia. J Alzheimers Dis 20 Suppl 2: S293–310.

26. Apabhai S, Sornjan S, Sutton L, E1231 T, et al. (2011) Habitual physical activity in mitochondrial disease. PLoS One 6: e22294.

27. Bell AJ, Bhat MS (1992) Prevalence of overweight and obesity in Down’s syndrome and other mentally handicapped adults living in the community. J Intellect Disabil Res 36 (Pt 4): 359–364.

28. Taylor DJ, Kemp GJ, Radda GK (1994) Bioenergetics of skeletal muscle in Down’s syndrome. J Physiol 481: 121–152.

29. Taylor DJ, Radda GK (1994) Bioenergetics of skeletal muscle in Down’s syndrome. J Physiol 481: 121–152.

30. Lai F, Williams RS (1989) A prospective study of Alzheimer disease in Down syndrome. Arch Neurol 46: 849–853.

31. Moreira PI, Cardoso SM, Santos MS, Oliveira CR (2006) The key role of mitochondrial function in Down syndrome. J Ment Defic Res 50: E153–161.

32. Lai F, Williams RS (1989) A prospective study of Alzheimer disease in Down syndrome. Arch Neurol 46: 849–853.

33. Hansson Petersen CA, Alikhani N, Behbahani H, Wiehager B, Pavlov PF, et al. (2011) Accumulation of beta-amyloid precursor protein gene using adenovirus vector causes mitochondrial dysfunction in Down’s syndrome. J Biol Chem 286: 2621–2635.

34. Lee SH, Lee S, Jeong HJ, Cha WT, et al. (2003) Expression of the mitochondrial ATPase gene and Tiam in Down syndrome. Mol Cells 13: 101–105.

35. Joachim CL, Mori H, Selkoe DJ (1989) Amyloid beta-protein deposition in synaptic terminals and the amyloid beta-protein gene using adenovirus vector causes mitochondrial dysfunction in vivo. J Biol Chem 264: 18504–18510.

36. Busciglio J, Pelsman A, Wong C, Pagios G, Yuan M, et al. (2002) Altered metabolism of the amyloid beta precursor protein is associated with mitochondrial dysfunction in Down’s syndrome. Neurosurg Focus 13: 1–15.

37. Kim SH, Vilkonsky R, Cairns N, Lubec G (2000) Decreased levels of complex III core protein 1 and complex V beta chain in brains from patients with Alzheimer’s disease and Down syndrome. Cell Mol Life Sci 57: 1810–1816.

38. Lee SH, Lee S, Jun HS, Jeong HJ, Cha WT, et al. (2003) Expression of the mitochondrial ATPase gene and Tiam in Down syndrome. Mol Cells 13: 101–105.

39. Lytle ME, Vander Bilt J, Pandav RS, Dodge HH, Ganguli M (2004) Exercise training improves functional outcome and cognitive decline: the MoVIES project. Alzheimer Dis Assoc Disord 18: 57–64.