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What effect does regular exercise have on oxidative stress in people with Down syndrome? A systematic review with meta-analyses

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Abbreviations: ROS, reactive oxygen species; SOD, superoxide dismutase; GPX, glutathione peroxidase; CAT, catalase; Aβ, amyloid-beta; BMI, body mass index; GSH, reduced glutathione**;** TBARS= thiobarbituric acid reactive substances; MDA= malondialdehyde; GSSG= oxidised glutathione; LOOH= lipid hydro-peroxide; SMD, standardised mean difference; CI, confidence interval; RCT, randomised controlled trial;

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Abstract

Objective: What effect does regular exercise have on oxidative stress in people with Down syndrome?

Design: Systematic review with meta-analyses.

Methods: A systematic review with meta-analyses was conducted. Six databases were searched from inception until August 2017. Studies where included if participants with Down syndrome (any age) had completed an exercise program of at least 6 weeks duration and at least one biomarker measured the generation or removal of reactive oxidative species. Data were extracted using a customised form. Risk of bias was assessed using the Cochrane Collaboration's Risk of Bias assessment tool. Effect sizes were calculated and meta-analyses completed for clinically homogeneous data using a random effects model.

Results: Seven studies (11 articles) involving 144 inactive participants investigated the effect of moderate intensity aerobic exercise. No pattern emerged for how most biomarkers responded with non-significant pooled effect sizes and high levels of heterogeneity observed. The exception was catalase which increased significantly after exercise (standardised mean difference 0.39, 95%CI 0.04 to 0.75; I^2 15%). Available studies were at high risk of bias. Two of five studies that measured more than one biomarker reported a decrease in oxidative stress with increased antioxidant activity after exercise but the other three (including one small randomised controlled trial) reported increased oxidative stress with variable change in antioxidant activity.

Conclusions: There remains uncertainty about the effect of exercise on oxidative stress in people with Down syndrome.

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Keywords: Trisomy 21; Physical activity; Reactive oxygen species; Antioxidants; Disability

Introduction

Down syndrome (trisomy-21) is the most common known cause of intellectual disability.[1] It has whole-of-genome and epigenetic effects with consequences for the structure and function of every organ system. The intellectual disability is usually mild or moderate with variation in ability to manage daily living activities, and physical impairments include poor cardiovascular fitness.[1] Improvements in early cardiac care for children with Down syndrome have increased life expectancy[2] resulting in a growing adult population.[3] However, this population is not ageing well.[4] Half to three quarters of adolescents and adults with Down syndrome are overweight or obese.[5,6] Older adults with Down syndrome have high rates of age related morbidity and most experience early cognitive decline with a cumulative risk of dementia of 45% by 55 years and 80% by 65 years[7] compared to 20 to 35% by 75 years in the general population.

Oxidative stress is defined as the imbalance between the generation and removal of reactive oxygen species (ROS) in the body and is elevated from birth in people with Down syndrome.[8] It is caused by the overexpression of superoxide dismutase-1 (SOD1), encoded by the SOD1 gene located on chromosome 21.[8] SOD catalyses the dismutation of the superoxide anion to hydrogen peroxide which is then converted to water by glutathione peroxidase (GPX) and catalase (CAT). In Down syndrome, the ratio of SOD to GPX and CAT is increased,[9] producing more hydrogen peroxide than CAT and GPX can catabolise. The excess hydrogen peroxide and/or its conversion product (hydroxyl radical), can lead to cellular oxidative damage.[9]

The consequences of oxidative stress include neurodegeneration and intracellular accumulation of amyloid-beta (Aβ) deposits (that define Alzheimer disease),[10] which have a direct role in the cognitive decline in Down syndrome.[4, 11, 12] The brain is particularly susceptible to oxidative stress because of its high lipid content. In Down syndrome, increased ROS production renders neurons prone to apoptosis and more likely to degenerate.[10] Adults with Down syndrome have oxidative damage in the brain prior to the onset of Aβ deposits.[13] Further, the oxidative system is associated with cognitive

functioning in adults with Down syndrome.[14] Increased lipid peroxidation[12] and poorer SOD functioning predicts poorer memory functioning in older adults with Down syndrome.[15]

Regular exercise reduces oxidative stress and enhances antioxidant activity in the general population[16] including the elderly.[17, 18] The effects in people with Down syndrome are unclear because their physiological responses to exercise differ from the general population.[19] The physiological responses to exercise of people with Down syndrome include diminished cardiac responses, blunted arterial stiffness responses, autonomic dysfunction and chronotropic incompetence, each contributing to reduced exercise capacity, limited work performance and poorer exercise economy compared to healthy controls.[19] Studies also show differences in response to *a single session of aerobic exercise* in oxidative stress and antioxidant activity[20, 21] between people with and without Down syndrome. Oxidative stress decreased[21] or did not change[20] in young adults with Down syndrome, whereas in healthy controls, there was an increase in oxidative stress immediately after exercise followed by a decrease to resting values after 60 minutes of recovery.[20] There were also between group differences for antioxidant activity. Immediately after a single exercise session and during recovery total antioxidant capacity decreased in healthy controls, but did not change in young adults with Down syndrome.[20]

Given the different responses to a single session of exercise, we cannot assume people with Down syndrome will respond in terms of oxidative stress in the same way to regular exercise as the general population. Therefore, we completed a systematic review and conducted meta-analyses on clinically homogeneous measures to investigate the effect of regular exercise on oxidative stress in people with Down syndrome.

Method

This systematic review was reported with reference to the PRISMA guidelines[22] and was prospectively registered with PROSPERO (CRD42016048492). Six electronic databases (Medline, EMBASE, CINAHL, PubMed, AMED, SPORTDiscus) were searched from inception to August

2017. The search strategy covered three main concepts: Down syndrome, exercise and oxidative stress, along with synonyms of each (Supplemental file 1). The search yields were downloaded into Endnote bibliographic software (version X7) and duplicates were removed. A manual search of the reference lists of included studies was also performed and citation tracking of the included articles was completed using Google Scholar.

Studies were included in the review if (1) the participants had been diagnosed with Down syndrome (any age), (2) exercise training or physical activity (all types) was completed for at least 6 weeks, (3) at least one measure of oxidative stress was included, (4) written in English and (5) available in full text. The exercise or physical activity interventions could include but were not limited to aerobic training, strength training, walking, swimming or cycling. The setting for the intervention could be at home, in a laboratory or at a community venue. All quantitative study designs (e.g. pre-test/post-test intervention designs, controlled trials) that assessed any biomarker measuring outcomes related to the generation of reactive oxygen species (oxidative stress), its removal and products of reactions between reactive oxygen species and lipid or protein biomolecules over any time-points were included.

Studies were excluded if (1) data from participants with Down syndrome were included as part of a larger group of participants (e.g. people with intellectual disability) but their data could not be separated from the larger cohort; (2) the effect of a single session of exercise on oxidative stress was investigated; (3) they were qualitative studies or narrative reviews. Two reviewers (NS, NT) independently assessed the titles and abstracts of the search yield for eligible articles based on the criteria above. Full text versions of articles that could not be excluded based on title and abstract were obtained and the eligibility criteria reapplied. Reasons for exclusion were recorded. Any discrepancies were settled by discussion until consensus was reached.

Data were extracted on the following variables: study design, participant characteristics (age, sex, BMI, severity of intellectual disability, exercise participation), sample size (number of participants in

each study arm), intervention (including mode of exercise, intervention duration, frequency per week, individual session duration, setting, supervision required, exercise intensity), outcomes measured, statistical analysis and adverse events. Data were extracted by one reviewer (NS) using a standardised data extraction form developed for the review and checked by a second reviewer (MK). Any disagreements were discussed until consensus was reached.

Risk of bias was assessed independently using the Cochrane Collaboration's Risk of Bias assessment tool[23] by two reviewers (JD, HL). Seven domains were assessed using this two-part tool: sequence generation, allocation concealment, blinding of participants and personnel, blinding of outcome assessment, incomplete outcome data, selective outcome reporting and 'other issues'. The first part of the tool described what was reported to have happened in the study, in sufficient detail to support a judgment about the risk of bias. The second part of the tool assigned a judgment relating to the risk of bias for that domain. The judgment assigned by the reviewers for each domain for each study was either low risk of bias, high risk of bias, or unclear risk of bias. If insufficient detail was reported of what happened in the study, the judgment assigned was 'unclear risk' of bias. Disagreements were resolved through discussion until a consensus was reached.

Data on participant characteristics and the interventions implemented were synthesised descriptively. Effect sizes were calculated for all studies, where appropriate, using standardised mean differences of post intervention scores between experimental and control groups,[24] or pre and post intervention scores for pre-test/post-test studies estimated by^{-}, with associated 95% confidence intervals.[25]

A random effects meta-analysis, based on DerSimonian and Laird method,[26] was completed for clinically homogeneous measures. Data were considered clinically homogeneous if the same design (e.g. pre-test/post-test) was used to evaluate the same or related biomarkers in the same population. Heterogeneity among studies was evaluated by the I^2 statistic, with values below 25% indicating low heterogeneity, 25-75% moderate heterogeneity, and more than 75% high heterogeneity.[27]

Heterogeneity was further investigated by running a meta-regression that investigated the effect of

measured covariates (i.e., age, year of study) on the observed heterogeneity in change in biomarker levels across the studies. Only pre-test/post-test studies in which the effect sizes were possible to estimate from known *t*-scores were included in the meta-analyses. Assessment of publication bias was estimated using a funnel plot. Secondary analyses comparing the outcomes of studies that involved the following comparisons: (1) exercise with usual care or no exercise, and (2) different forms of exercise (e.g. aerobic versus strength training exercise) were planned, but not conducted due to insufficient data.

Results

Electronic database searching and citation tracking identified 159 studies with 99 remaining after the removal of duplicates (Supplemental file 2). After the application of the eligibility criteria, 11 articles [28-38] reporting outcomes from seven studies [28, 30, 32, 34, 35, 37, 38] were included in the review (Table 1). Five articles[29-31, 33,36] reported outcomes from the same set of participants; Ordonez et al [30] was the first of these to be accepted for publication and so for the purposes of this review was used to reference this study hereafter. Study designs included one randomised controlled trial,[35] one pre-test/post-test study with control data for some outcomes,[30] and five single group pre-test/post-test studies.[28, 32, 34, 37, 38] There was moderate agreement between reviewers when applying eligibility criteria to titles and abstracts (κ= 0.68 95% CI 0.42 to 0.90).

Seven studies with 144 participants (142 male, 2 female) were included. The weighted mean age of participants was 18.4 years, ranging from 14.9 to 23.3 years (table 1). The weighted mean BMI was 23.9 kg/m² based on data reported in five studies. The severity of intellectual disability was reported in three studies as mild to moderate,[37] moderate [35] and moderate to severe.[38] Five studies[28, 29, 31, 37, 38] specified that participants were inactive or had not engaged in exercise in the previous six to 12 months. One study[38] reported cardiac status and one study[32] reported on absence of atlanto-axial instability.

[Insert Table 1 about here]

All studies investigated the effect of aerobic exercise (Table 2). Participants trained on either treadmills or cycle ergometers in four studies,[28, 30, 34, 38] performed walk/run training on an indoor track in one study,[37] and completed adapted judo training in one study.[32] One study[35] did not report the type of training. The average duration of intervention was 12.3 weeks (ranging from 6 to 16), with participants exercising on average 3 times per week (ranging from 3 to 5 times per week). Exercise sessions lasted on average 62 minutes (ranging from 40 to 120 minutes including warm up and cool down phases), with the active phase of training being 34 minutes on average. The exercise intensity was described in five studies[28, 30, 32, 35, 38] as moderate (i.e. 60-75% peak heart rate or peak oxygen uptake) and was not reported in the other two studies.[34, 37] Four studies described how training was progressed. Two studies[28, 35] progressed exercise intensity by increasing training time; one study[34] increased treadmill speed, incline and time over four weeks, then kept the intensity constant for eight weeks; and one study[30] increased time and intensity every three weeks for 12 weeks. One study[37] reported participants trained in groups but did not report group size. The other studies did not report if participants trained individually or in a group. None of the studies reported who supervised the training sessions. Only three studies reported where the training was performed: a combination of at school and in a laboratory;[28] at a university[34] and on an in-door athletic track on a university campus.[37]

[Insert Table 2 about here]

Only one study[32] provided information on intervention fidelity; blood lactate monitoring showed exercise was performed at moderate intensity below anaerobic threshold. Two studies[29, 38] reporting using heart rate monitors during training but did not report data from monitoring. Only one study[32] reported data on attendance, which was above 80%. The same study was the only one to report data on adverse events and noted no severe mechanical injuries among the participants.[32] Drop-out rates of participants receiving an exercise intervention were not reported in five studies;[29, 32, 35, 37, 38] there were no drop outs in one study,[34] but in another study[28] of 11 participants who agreed to participate only six completed the program.

A summary of the risk of bias assessment for each study is presented in Supplemental file 3. Six studies assessed a single group before and after the exercise intervention[28, 30, 32, 34, 37, 38] and therefore lacked any mechanism to reduce allocation bias. One study assessed two groups for comparison using randomisation for group allocation but did not describe the process.[35] One study [31] included some control data for two outcomes but did not use randomisation. Allocation bias was therefore high for all studies. Researchers and participants were not blinded to procedures in all studies, but the risk of performance and detection bias was rated as low because blood levels of ROS would be unlikely to be influenced by this knowledge. Two studies reported high attrition fractions (7/11[28] and 5/15[38]) suggesting a high risk of attrition bias. The remaining studies did not report dropouts and attrition bias was rated as unclear. One study[33] did not report data for the control group suggesting a high likelihood of reporting bias. Reporting bias is not known for the other studies. Sampling bias was likely to be very high in each of the studies. Sample sizes were small and were unlikely to be representative of the breadth of phenotype in Down syndrome. For example, only one study recruited females.[28] There was limited reporting of the category of intellectual disability, comorbidities and levels of physical activity.

Biomarkers of oxidative stress measure outcomes related to the generation of ROS (i.e. lipid peroxidation, protein oxidation) and the removal of ROS (i.e. antioxidant enzymes). Of the studies included, two[28, 38] measured one biomarker of oxidative stress only; in both studies the biomarker was an antioxidant (SOD, an antioxidant enzyme and GSH respectively). The other five studies measured at least two biomarkers, including at least one biomarker measuring outcomes related to the generation of ROS (e.g. TBARS, MDA, carbonyls) and at least one biomarker measuring the removal of ROS (e.g. SOD, GPX, CAT, GSH).

When data were compared across studies, no clear pattern emerged from either descriptive or meta-analyses for how most individual biomarkers responded to regular exercise in people with Down syndrome (Supplemental file 4). Different studies showed opposite outcomes for the same biomarkers. For example, two studies[30, 32] measured protein oxidation via carbonyls. Of these, one

study[30] reported a large decrease in carbonyls, while the other[32] reported a moderate increase in carbonyls after exercise. Statistically insignificant pooled effects sizes, with high levels of heterogeneity (I^2>80%), were observed for four out of the five biomarkers (i.e., TBARS, MDA, SOD, and GPX) investigated (Figure 1). The exception was catalase which increased significantly after exercise (SMD 0.39, 95% CI 0.04 to 0.75; I^2 15%). However, the available body of evidence was characterised by a high risk of bias and a probable publication bias as judged by a visual inspection of a funnel plot showing asymmetry suggesting a higher probability of publication of studies with either a negative or positive significant outcome (Supplemental file 5). Random effects meta-regression showed that age significantly accounted for just over one third (36%) of the observed heterogeneity (Adjusted R^2 = 0.361, p = 0.008).

[Insert Figure 1 about here]

Given there was no clear pattern in how biomarkers of oxidative stress in people with Down syndrome responded to exercise, the biomarkers measured in the five studies that included more than one outcome were also interpreted relative to each other. Two studies[30, 34] reported a decrease in one biomarker measuring outcomes related to the generation of ROS and an increase in one biomarker measuring antioxidant activity after regular exercise (Supplemental file 4). However, the other three studies[32, 35, 37] (including one small RCT) reported an increase in biomarkers measuring outcomes related to the generation of ROS (TBARS, MDA, carbonyls, LOOH) in people with Down syndrome after exercise. In these three studies, the associated change in the antioxidant system varied. For example, Monteiro et al[35] reported a decrease in GSSG, an increase in GSH and no change in SOD which suggested some adaptation to exercise as GSSG was converted to GSH with no associated change in antioxidant enzymes. Aguiar et al[32] reported a concurrent increase in the antioxidant enzymes SOD and CAT but no change in GPX, again suggesting some adaptation to exercise occurred. Lastly, data reported by Ozbey et al [37] suggested a decrease in both SOD and GPX and no change in CAT.

Discussion

The main finding of this review was that no clear pattern emerged for how individual biomarkers of oxidative stress or antioxidant activity responded to regular short term aerobic exercise programs in people with Down syndrome. People with Down syndrome generally participate in low levels of physical activity and exercise[39, 40] but those who experience less cognitive decline have lower oxidative stress,[12] and may have engaged in more physical activity. These uncertainties need to be resolved in order to understand more comprehensively the biological and functional benefits of regular exercise to people with Down syndrome.

The high risk of bias of the included studies in this review provides an explanation for the uncertain findings. All included studies, with one exception, used single group pre-test/post-test designs which are subject to bias and can overestimate the size of any effect.[41] Available control data were minimal and also of high risk of bias; the included randomised controlled trial did not use concealed allocation or blinded assessment and the only other available control data (7 participants) presumably were collected after completion of the pre-test/ post-test trial.[35] In addition, the choice of biomarkers to measure oxidative stress and antioxidant activity may have been a source of error and obscured any true effects of exercise for people with Down syndrome. For example, TBARS and MDA are now considered less accurate markers of oxidative stress[42] and newer, more specific probes to measure outcomes related to ROS production such as L012 (superoxide),[43] DCFDA (hydrogen peroxide),[44] urinary isoprostanes[45] and oxidised DNA bases[46] provide better indications of ROS levels.[47] Also, some included studies only measured a single biomarker,[28, 38] which makes the data difficult to interpret. For example, an increase in SOD could be positive if associated with a concurrent increase in GPX and CAT. This makes it difficult to interpret the meta-analysis of a single biomarker, CAT, which reached statistical significance. The high risk of bias in study designs plus limitations in the choice of biomarkers may explain the variation in reported results.

Sample sizes were small (ranging from 4 to 38 participants) and resulted in large confidence intervals around the estimate of effect. For example, the estimates of effect for the antioxidant activity of SOD in the controlled study ranged from -0.91 to 1.05, that is, with 95% confidence there could possibly have been a large effect that SOD was either reduced or increased in response to regular exercise.[35] The studies were also not representative of the breadth of phenotype in Down syndrome. Only one included study recruited females[28] and only two included studies reported co-morbidities.[32, 38] These sampling biases limit the generalisability of findings.

There is a need to resolve the issue of whether regular exercise has a positive or negative effect on oxidative stress for people with Down syndrome across the breadth of the phenotype. This will enable development of strategies to improve the health of people with Down syndrome, specifically to address the consequences of excessive oxidative stress such as accelerated ageing, neurodegeneration, impaired cognitive functioning and dementia.[10, 13, 48] If regular exercise has a positive effect on oxidative stress, it would strengthen the rationale to support efforts to promote physical activity, including regular (structured) exercise, in people with Down syndrome. In this case efforts would focus on strategies to increase exercise opportunities and promote exercise adherence.[49-51] However, if in fact, regular exercise has a negative effect on oxidative stress for people with Down syndrome, then exercise programs for people with Down syndrome may need to be tailored so that the known benefits of exercise in this group (improved strength, cardiovascular fitness, and social inclusion)[52-54] are retained without causing biological harm. There is strong evidence from a meta-analysis of 19 controlled trials that regular exercise decreases biomarkers of oxidative stress and increases anti-oxidant activity in people in the general population.[55] Similar evidence from adequately powered randomised controlled trials are required to resolve this question in Down syndrome and thereby inform recommendations regarding the type and dose of exercise that is most likely to be of biological beneficial to people with this condition.

The main strength of this systematic review was the rigorous methods used, which included prospective registration, detailed search strategies, evaluation of the risk of bias, meta-analyses and

reporting consistent with the PRISMA guidelines.[22] Available data are however limited and of a poor quality. However, the included studies provide preliminary data to highlight the importance of clarifying the effects on oxidative stress in people with Down syndrome, and whether regular exercise can ameliorate the effects of the secondary health conditions to which people with Down syndrome are vulnerable. Also, since the included studies in the meta-analyses followed a pre and post study design or a pre-experimental design without controls, they had little power to establish causality.

Conclusions

Our systematic review suggests there remains uncertainty about the effect of regular exercise on oxidative stress and antioxidant activity in people with Down syndrome. Since oxidative stress predates clinical pathology in people with Down syndrome, there is a need to resolve this uncertainty with adequately powered randomised controlled trials.

Practical implications

- Available studies on the effect of regular exercise on oxidative stress in people with Down syndrome are at high risk of bias.
- No clear pattern emerged for how most individual biomarkers of oxidative stress or antioxidant activity responded to short term aerobic exercise programs in people with Down syndrome.
- Data from pre-test/post-test studies showed catalase increases significantly after exercise in people with Down syndrome.

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453

Figure legend

Figure 1 Meta-analysis of data from pre-test/post-test studies using biomarkers of oxidative stress (A), CAT (B), GPX (C), SOD (D).

Table 1: Characteristics of the included studies

Study	Research design	Sample size	Age (yrs)	Sex	BMI (kg/m²)	Severity of intellectual disability	Previous exercise participation	Oxidative stress biomarkers	Antioxidant biomarkers	Adverse events
Aguiar et al **[32]**	Single group pre-test/ post-test	21	23.3±2.1	21M	23.0±1.2	Not reported	Not reported	TBARS (E&S) lipid hydro-peroxides (S) carbonyl (E&S)	SOD (E&S) CAT (E&S) GPX (S)	No severe mechanic al injuries
Aleksander-Szymanowicz et al **[38]**	Single group pre-test/ post- test	15	22.4±0.9	15M	27.5 (no SD reported)	Moderate or severe	"Non-trained"		GSH (E)	Not reported
Eberhard et al **[28]**	Single group pre-test/ post- test	4	18.4±1.8	2M, 2F	Not reported	Not reported	Inactive; 1hr physical education class per week		SOD (E)	Not reported
Meguid et al **[34]**	Single group pre-test/ post- test	30	16.6±1.1	30M	27.7±1.9	Not reported	Not reported	MDA (pl)	GPX (E)	Not reported
Monteiro et al **[35]**	RCT	16 (8 per group)	21.3±2.8	16M	Not reported	Moderate (IQ 39±9.7)	Not reported	TBARS (pl) GSSG (E & pl)	GSH (E & pl) SOD (E)	Not reported
Ozbey et al **[37]**	Single group pre-test/ post- test	20	14.9±7.1	20M	31.9±6.8	IQ 40-60	None for previous 12 months	MDA (pl) β-carotene (pl) Vit A (pl)	SOD (E) GPX (E) CAT (E)	Not reported
Ordonez et al **[30]** Ordonez et al **[29]** Rosety-Rodriguez et al **[33]** Ordonez et al **[31]** Ordonez et al **[36]**	Single group pre-test/ post- test with control data for 2 biomakers*	31 (+7 control for 2 outcomes)	16.3±1.1	31M	29.3±1.3	Not reported	None in previous 6 months	Carbonyl (pl)* MDA (pl)* uric acid (pl) allantoin (pl)	GPX(E) SOD(E)	Not reported

Note: *This study included control data from 7 males participants for two biomarkers only (carbonyl and MDA). These 5 papers all report outcomes from the same study. Two papers provide data from a n=7 control group. E=erythrocyte; Pl= plasma; S= serum; RCT= randomised controlled trial; yrs= years; BMI= body mass index; M= male; F= female; SD= standard deviation; Ex= exercise

Table 2: Overview of the intervention protocols

Study	Exercise type	Intervention (exercise mode)	Length of program (weeks)	Frequency (per week)	Intensity	Duration (of each session in minutes)	Progression	Individual or Group	Supervision (ratio and training of supervisor)	Location (e.g. clinic, gym, laboratory)	Materials used (e.g. heart rate monitors)	Adherence (intervention intensity fidelity)	Attendance (number of sessions attended)
Aguiar et al [32]	Aerobic	Adapted judo training	16	3	Moderate (monitored by lactate threshold)	50	Not reported	Not reported	Not reported	Not reported	Blood lactate monitoring	Blood lactate levels show exercise performed below anaerobic threshold	Above 80%
Aleksander-Szymanowicz et al [38]	Aerobic	Cycle ergometer	6	3	Moderate 60-75% peak heart rate	10 minute warm up, 20-25 minute main phase, 10 minute cool down	Not reported	Not reported	Not reported	Not reported	Heart rate monitor	Not reported	Not reported
Eberhard et al [28]	Aerobic	Activities (games with walking & running x 1hr) + cycle ergometer (twice a week)	12	School activities daily, cycle ergometer 2	Moderate (60% VO_2 max)	"up to 2 hours"	↑ time until participants could do continuous exercise for 60 mins	Not reported	Not reported	School and laboratory	Not reported for training	Not reported	Not reported
Meguid et al [34]	Aerobic	Treadmill	12	3	Not reported	10-40 (included warm-up, active training, cool down phases)	Time speed and incline increased gradually over 4 weeks, then constant for 8 weeks	Not reported	Not reported	University	Not reported	Not reported	Not reported

Monteiro et al [35]	Aerobic	Not reported	16	3	Moderate (60-75% VO_2 peak)	10 mins warm up, 15-25 mins active training, 5 mins cool down	Increase session 5 mins every 5 weeks	Not reported	Not reported	Not reported	Not reported	Not reported	Not reported
Ozbey et al [37]	Aerobic	Run/walk	12	3	Not reported	10-15 mins warm up, 30 minute run/walk, 10-15 mins cool down	Not reported	Group (number not reported)	Not reported	In-door athletic track on university campus	Not reported	Not reported	Not reported
Ordonez et al [29-31, 36] Rosety-Rodriguez et al [33]	Aerobic	Treadmill	12	3	Moderate (60-75% peak HR)	10-15 mins warm-up, 20-35 minute active training part, 10 min cool down	Increase duration of active training by 5 min every 3 weeks; increased intensity by 5% every 3 weeks	Not reported	'Supervised by researchers'	Not reported	Heart rate monitor	Not reported	Not reported

Figure

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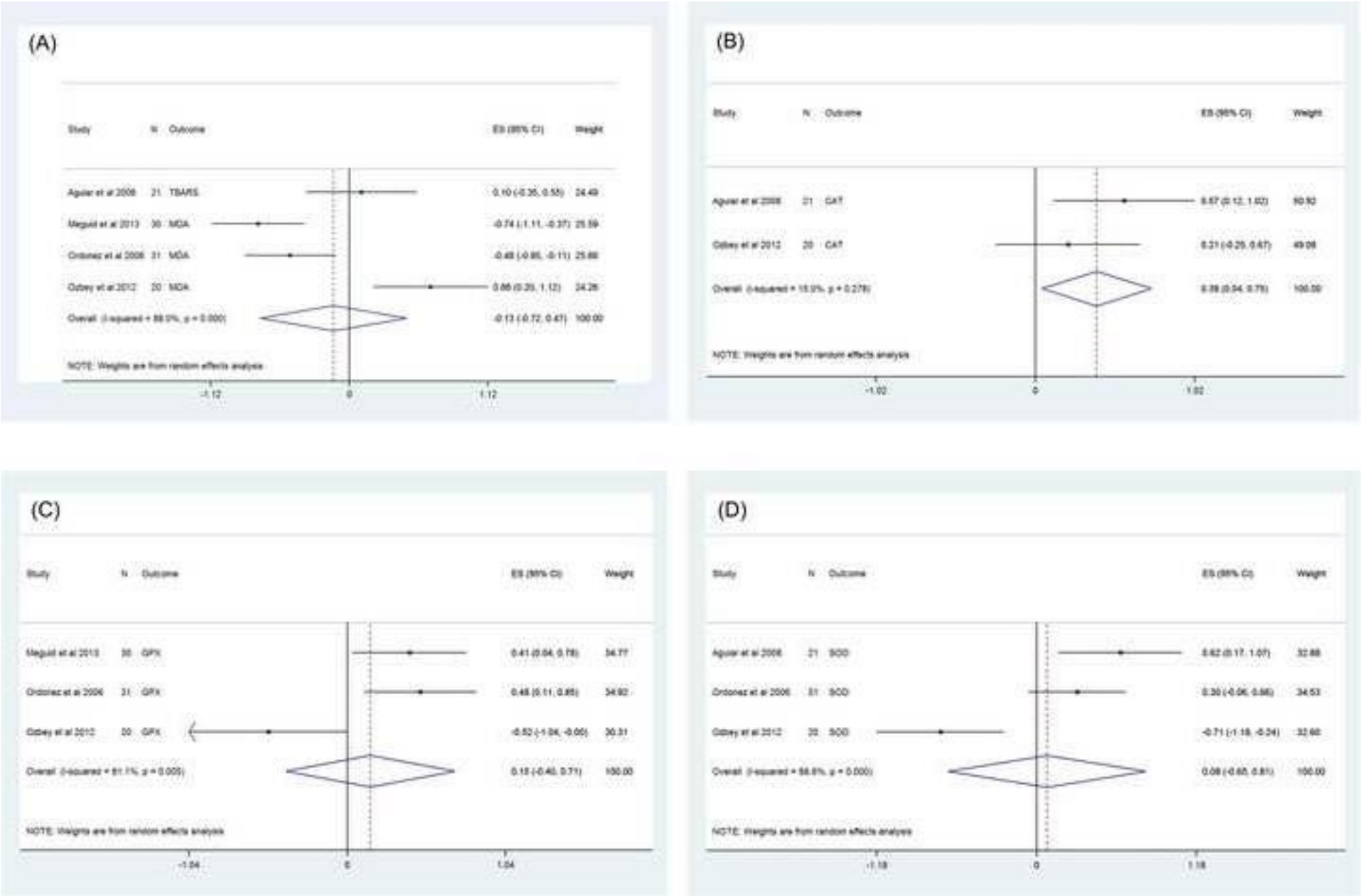
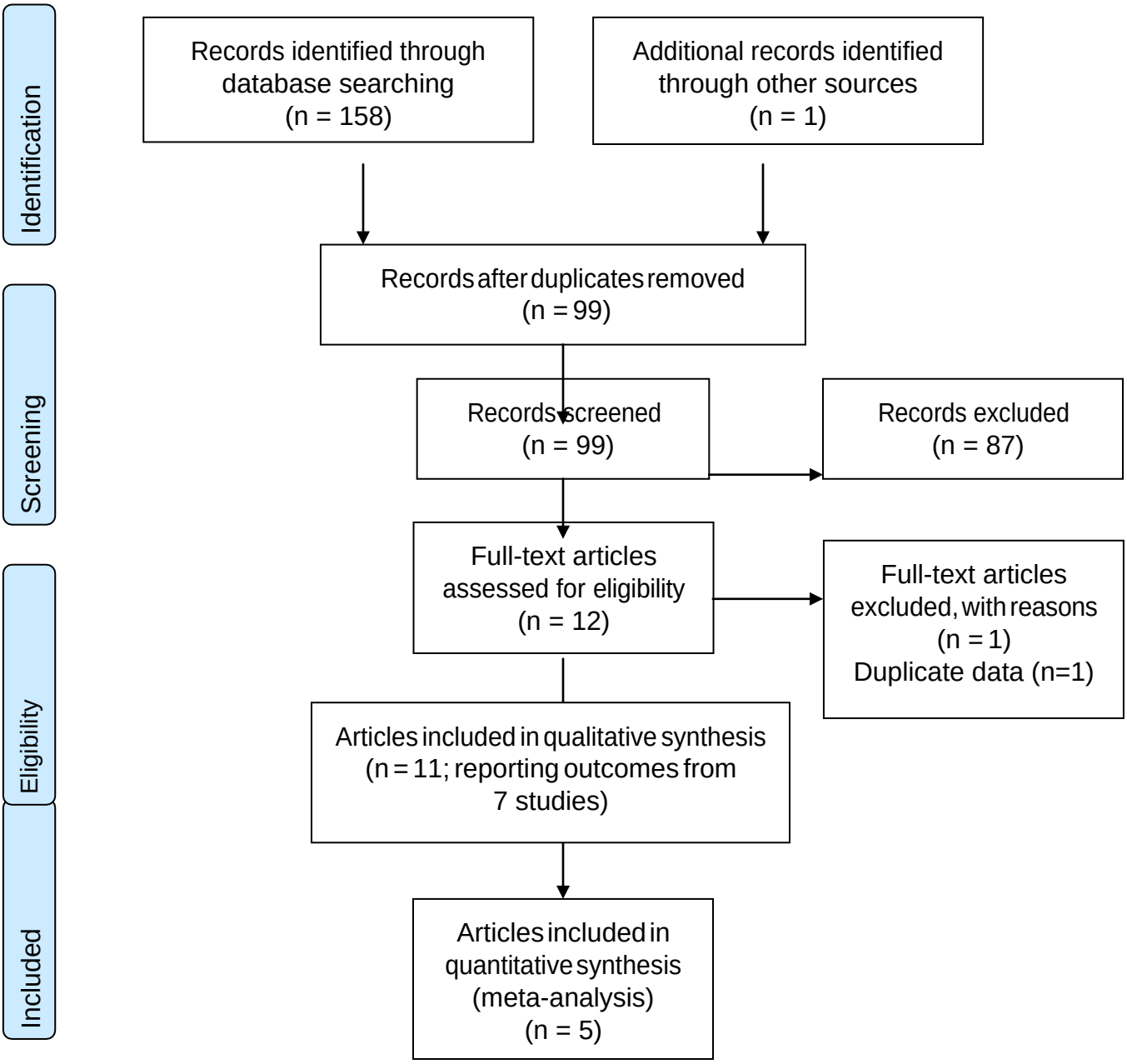
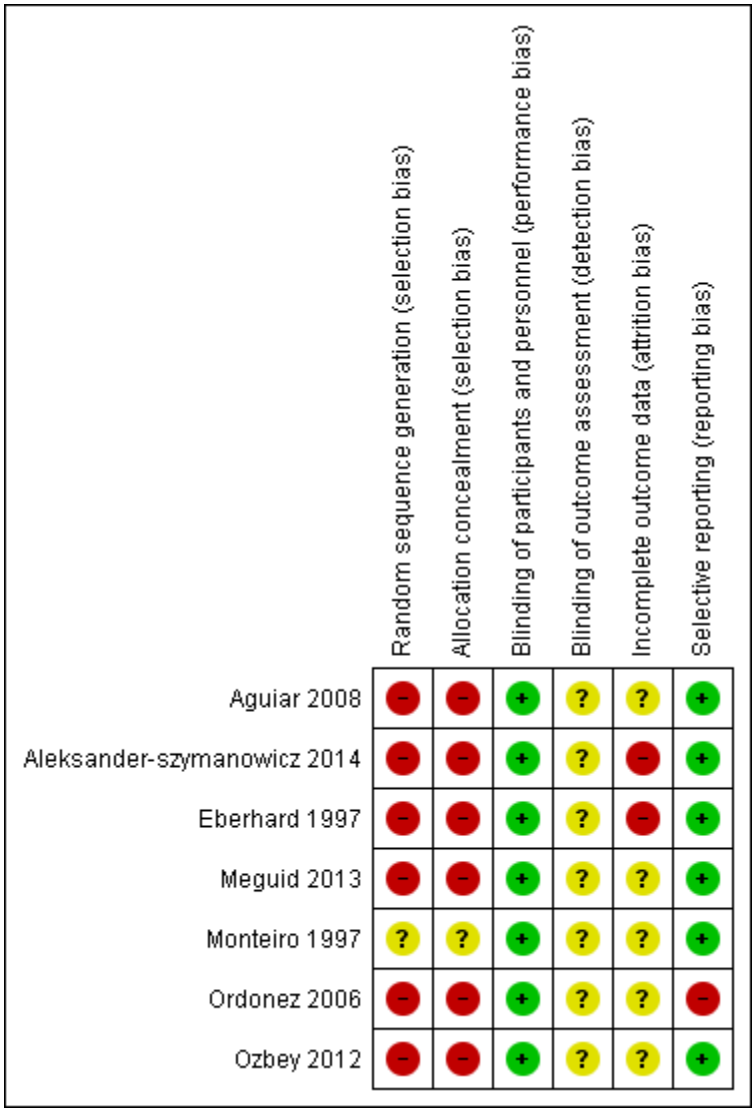


Supplemental file 1: Search strategy

1. Down Syndrome/
2. Intellectual Disability/
3. Down syndrome.ti,ab.
4. intellectual disability.ti, ab.
5. Trisomy 21.ti, ab.
6. mental retardation.ti,ab.
7. intellectual impairment.ti,ab.
8. 1 or 2 or 3 or 4 or 5 or 6 or 7
9. Exercise/ or Exercise Therapy/
10. Motor Activity/
11. exercise.ti,ab.
12. physical activity.ti,ab.
13. exercise therap$.ti,ab.
14. physical exercise.ti,ab.
15. physical training.ti,ab.
16. aerobic training.ti,ab.
17. aerobic exercise.ti,ab.
18. physical fitness.ti,ab.
19. progressive resistance training.ti,ab.
20. strength training.ti,ab.
21. weight training.ti,ab.
22. anaerobic exercise.ti,ab.
23. therapeutic exercise.ti,ab.
24. resistance training.ti,ab.
25. 9 or 10 or 11 or 12 or 13 or 14 or 15 or 16 or 17 or 18 or 19 or 20 or 21 or 22 or 23 or 24
26. Oxidative Stress/
27. Reactive Oxygen Species/
28. oxidative stress.ti,ab.
29. reactive oxygen species.ti,ab.
30. oxidative damage.ti,ab.
31. Antioxidant defense.ti,ab.
32. malondialdehyde.ti,ab.
33. pro-oxidant$.ti,ab.
34. superoxide dismutase.ti,ab.
35. 26 or 27 or 28 or 29 or 30 or 31 or 32 or 33 or 34
36. 8 and 25 and 35



Supplemental file 2: PRISMA flowchart for study selection



Supplemental file 3: Summary of the risk of bias assessment

Supplemental file 4: Biomarkers of oxidative stress and anti-oxidant enzymes and their response to regular exercise in people with Down syndrome

Biomarker	Sample type	Study	Sample size	Result	Effect size (95% CI)
Antioxidant					
SOD	Erythrocyte	Monteiro et al [35]*	16	No change in SOD	0.07 (-0.91 to 1.05)
	Erythrocyte	Aguiar et al [32]	21	↑ SOD	0.62 (0.16 to 1.07)
	Erythrocyte	Ozbey et al [37]	20	↓ SOD	-0.71 (-1.18 to -0.24)
	Erythrocyte	Ordonez et al [30]	31	No change in SOD	0.30 (-0.05 to 0.67)
	Erythrocyte	Eberhard et al [28]	4	No change in SOD	NA
	Serum	Aguiar et al [32]	21	No change in SOD	0.12 (-0.32 to 0.58)
GPX	Erythrocyte	Meguid et al [34]	30	↑ GPX	0.41 (0.04 to 0.79)
	Erythrocyte	Ozbey et al[37]	20	↓ GPX†	-0.52 (-0.99 to 0.05)
	Erythrocyte	Ordonez et al [29]	31	↑ GPX	0.48 (0.11 to 0.85)
	Serum	Aguiar et al [32]	21	No change in GPX	0.12 (-0.33 to 0.57)
CAT	Erythrocyte	Ozbey et al [37]	20	No change in CAT	0.21 (-0.25 to 0.68)
	Erythrocyte	Aguiar et al [32]	21	↑ CAT	0.57 (0.12 to 1.03)
	Serum	Aguiar et al [32]	21	↑ CAT†	0.34 (-0.11 to 0.79)
GSH	Plasma	Monteiro et al [35]*	16	↑ GSH	1.85 (0.68 to 3.02)
	Erythrocyte	Monteiro et al [35]*	16	No change in GSH	-0.74 (-1.76 to 0.27)
	Erythrocyte	Aleksander-Szymanowicz et al [38]	15	↑ GSH	1.07 (0.51 to 1.62)
Lipid peroxidation					
TBARS	Plasma	Monteiro et al [35]*	16	↑ TBARS†	0.93 (-0.1 to 1.96)
	Plasma	Aguiar et al [32]	21	No change in TBARS	0.10 (-0.35 to 0.55)
	Erythrocyte	Aguiar et al [32]	21	↑ TBARS	0.59 (0.13 to 1.04)
MDA	Plasma	Ordonez et al [31]	31	↓ MDA	-0.48 (-0.85 to -0.11)
		Meguid et al [34]	30	↓ MDA	-0.74 (-1.12 to -0.37)
		Ozbey et al [37]	20	↑ MDA	0.66 (0.20 to 1.13)

Protein oxidation					
Carbonyl	Plasma	Ordonez et al [31, 36]*	38	↓ carbonyls	-7.93 (-9.89 to -5.97)
	Plasma	Aguiar et al [32]	21	↑ carbonyls	0.62 (0.16 to 1.07)
	Erythrocyte	Aguiar et al [32]	21	↑ carbonyls	0.58 (0.12 to 1.03)
Other outcomes					
LOOH	Plasma	Aguiar et al [32]	21	↑ LOOH	0.49 (0.04 to 0.95)
GSSG	Plasma	Monteiro et al [35]*	16	No change in GSSG	0.8 (-0.22 to 1.82)
	Erythrocyte	Monteiro et al [35]*	16	↓ GSSG	-4.32 (-6.11 to -2.53)
Uric acid	Plasma	Rosety-Rodriguez et al [33]	31	No change in uric acid	0.29 (-0.07 to 0.66)
Allantoin	Plasma	Rosety-Rodriguez et al [33]	31	↓ allantoin	-0.66 (-1.02 to -0.29)

NA= data unavailable to calculate an effect size; CI= confidence interval
SOD=superoxide dismutase; GPX= glutathione peroxidase; CAT= catalase; GSH= reduced glutathione; TBARS= thiobarbituric acid reactive substances; MDA= malondialdehyde; GSSG= oxidised glutathione; LOOH= lipid hydro-peroxide
* controlled study
† a significant change post intervention was reported in the original paper.

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