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Blood Lactate concentrations during rest and exercise in people with Multiple Sclerosis: A systematic review and meta-analysis

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Abstract

Background: Multiple Sclerosis (MS) is a chronic disorder which irreversibly damages axons within brain matter. Blood lactate concentration could be a biomarker of MS onset and progression, but no systematic review has yet sought to confirm or dispute the elevation and biomarker potential of blood lactate in people with MS (PwMS) or to consolidate understanding of lactate production during exercise in PwMS.

Objective: To perform a systematic review and meta-analysis on blood lactate in PwMS during rest and exertion compared to Healthy Controls (HC) and following chronic exercise intervention.

Methods: A systematic search of six electronic databases (PubMed, CINAHL, Science Direct, Cochrane Library, SPORTDiscus and PEDro) was performed on 10th April 2020. Mean, standard deviation and sample size for lactate measures at rest and during exercise were pooled to determine overall effect size using a random effects model. The 20-point Appraisal tool for Cross-Sectional Studies was utilised to assess study quality and inherent risk of bias. To qualify for inclusion, studies had to include human adults (>18 years) with a confirmed clinical diagnosis of MS, be published in English, have undergone peer review, report absolute blood lactate values for data extraction, and if involving testing during/after exercise, to do so during bilateral exercise methods.

Results: 18 studies were qualitatively analysed and 15 studies quantitatively analysed. Outcome data was available for 1986 participants (n_{MS} = 1129). A total of 7 papers tested blood lactate during rest ($Lactate_{REST}$), 7 papers tested during sub-maximal intensity exercise ($Lactate_{SUB\text{-}MAX}$), and 8 papers tested during maximal intensity exercise ($Lactate_{MAX}$). Meta analyses showed elevated $Lactate_{REST}$ and reduced $Lactate_{MAX}$ in PwMS compared to HC, higher $Lactate_{MAX}$ in lower EDSS-scoring PwMS compared to higher EDSS-scoring PwMS, and that $Lactate_{SUB\text{-}MAX}$ decreases and $Lactate_{MAX}$ increases in

PwMS following a chronic exercise intervention. Qualitative analysis reported $Lactate_{REST}$ to be reduced in PwMS following a chronic exercise intervention.

Conclusions: $Lactate_{REST}$ is elevated in PwMS compared to HC. $Lactate_{MAX}$ is lower in PwMS compared to HC and lower still in higher compared to lower EDSS-scoring groups of PwMS. Chronic exercise interventions have the potential to reduce $Lacatate_{SUB\text{-}MAX}$ for a given power output and increase $Lactate_{MAX}$ in PwMS compared to baseline values. $Lactate_{REST}$ may be reduced in PwMS following a chronic exercise intervention but more research is required for confirmation. The results of this review were limited by small sample sizes and number of studies available for each testing condition, limited data available for potentially confounding/correlating factors (eg. VO_2 and power output) as well as heterogeneity of methodology adopted across studies, often due to lactate testing being a secondary outcome measure.

PLS:

Lactate levels in the blood are different during rest and at intense exercise levels in people with Multiple Sclerosis (MS) compared to healthy counterparts, with people with MS showing a smaller jump in lactate during intense exercise from a higher resting level. After exercising for at least 3 months, blood lactate levels during exercise may become more similar to the levels seen in people without Multiple Sclerosis, but more research is required to give a clearer picture of this. We can hopefully use blood lactate in future to measure the progression of MS in an individual as well as the effectiveness of their exercise programme.

1.0 Introduction

Multiple Sclerosis (MS) is a chronic disorder characterised by perivenular inflammatory lesions which ultimately result in demyelinating plaques, oligodendrocyte damage, and irreversible damage of axons within the grey and white brain matter [1], resulting in a progressive loss of function across the spectrum of neurological control. Once diagnosed, the phenotype of MS will be established through the pattern of progression into one of 3 types: relapsing remitting (RR), primary progressive (PP), and secondary progressive (SP). Since revision of MS classifications in 2013, the term chronic progressive (CP) was dropped for the more specific PP and SP, and progressive relapsing (PR) was dropped for PP-active [2].The extent to which loss of function has occurred is measured by clinicians using the Extended Disability Status Scale (EDSS); an incremental rating from 0 (no observable symptoms) to 10 (death due to MS), with the total score consisting of a combination of the number and the severity of affected functional systems [3].

Lactate is the base form of lactic acid and is produced within the human body from the reversable reduction of pyruvate during anaerobic glycolysis within the cytoplasm of the cell [4]. Increases in lactate concentration occur during conditions of increased energy demand or reduced oxygen availability. Elevated lactate has been observed using MRI in acute plaques within brain tissue in patients with MS and is hypothesized to be a sign of affected mitochondrial function in MS or increased glycolysis during the inflammatory processes of demyelination [5], though precise causes are as yet unknown. There has also been evidence presented suggesting that PwMS present higher levels of lactate in blood plasma and cerebrospinal fluid at rest compared to healthy controls (HC) and in correlation with EDSS [6,7], cementing further the idea that elevated lactate results from progression of MS. There are a number of established and newly emerging biomarkers of MS in current research aiming to fulfil at least one of three functions: predicting an individual's risk of developing MS, monitoring progression of disease course, and monitoring responsiveness to specific

93 treatments in research or routine care [8]. Measurement of blood lactate can be performed via
94 catheterisation of arteries within the forearm but sampling at superficial capillarisation sites at the
95 fingertip and earlobe are the most common due to minimal invasiveness and close similarities with
96 arterial measurement [9]. With this minimal invasiveness and the relatively low cost of measurement
97 compared to other biomarkers, the above findings could suggest there is potential that blood lactate
98 could harbour potential as a simple and accessible biomarker for MS progression.
99
100 Lactate is typically present in healthy individuals at concentrations between 1-2mmol/L in the blood
101 during rest [10] and rises with increasing demand of rapid ATP production, resulting in a sharp increase
102 in concentration during higher exercise intensities [11]. When measured as part of an exercise-based
103 study, the moment during increasing exercise intensity at which the blood lactate concentration
104 undergoes an inflection point from a gradual to sharp increase is known as the lactate threshold [12],
105 while the work rate at which blood lactate concentration is 4mmol/L is defined as the onset of blood
106 lactate accumulation (OBLA) [13]. Lactate levels are often used as a measure of exercise intensity and
107 training performance due to relatively cheap and easy measurement and reasonable correlation with
108 anaerobic exercise intensity [14] and energy metabolism [15,16]. It has also been used to assess
109 exercise intensity and training response in PwMS [17–19]. However, such testing has generally been
110 performed in studies with relatively small sample sizes and limited statistical power. Understanding
111 the broader picture of how MS impacts lactate concentrations at sub-maximal and maximal intensity
112 exercises may help provide an overview of lactate dynamics in pwMS in daily living and during
113 strenuous activities. It could also indicate methodological suitability of lactate analysis for measuring
114 intervention and rehabilitation intensity and effectiveness for pwMS. This is particularly the case for
115 when PwMS are exercising within facilities with limited availability of ergometers for power-output
116 control and data recording, lacking clinicians for functional assessment, or should PwMS be
117 participating in home-based interventions or remote research during times of highly restricted ethical
118 research like those imposed during the onset of COVID-19 [20]. As such, a systematic review and meta-

119 analysis of full lactate response dynamics in PwMS is both warranted and overdue, given the potential
120 for both simple and cost-effective blood lactate testing and potential use as a biomarker for MS
121 progression. With this rationale, the objectives of this paper are to provide a systematic review and
122 meta-analysis of blood lactate concentrations during resting conditions and following acute maximal
123 intensity exercise bouts in PwMS compared to HC. Additionally, the paper will provide a systematic
124 review and meta-analyses of the changes in blood lactate responses in PwMS during submaximal and
125 maximal intensity exercise following chronic exercise interventions.
126
127 2.0 Methodology
128 2.1 Search Strategy
129
130 This systematic review was conducted was in accordance with the Preferred Reporting Items for
131 Systematic Reviews and Meta-Analyses (PRIMSA) statement [21]. A comprehensive search was
132 performed to find literature examining blood lactate in people with MS, either as a primary or a
133 secondary outcome. Six electronic databases (PubMed, CINAHL, Science Direct, Cochrane Library,
134 SPORTDiscus and PEDro) were searched on 10th April 2020. Search terms used were as follows:
135 “Multiple Sclerosis” AND (Lactate OR Lactic Acid OR OBLA OR glycolysis). Filters were applied where
136 possible so that only research articles and articles that were peer-reviewed would be retrieved.
137
138 2.2 Inclusion and Exclusion criteria
139
140 To be included into the review, the study had to include human adults (>18 years) with a confirmed
141 clinical diagnosis of MS; there were no limitations with respect to EDSS score or MS-type. Studies had
142 to have undergone peer review, be reported in English, report absolute blood lactate values for data
143 extraction, and, if involving exercise during lactate testing, had to be taken during bilateral exercise

methods (eg. Recumbent cycling, stepper or treadmill). In order to maximise the number of relevant studies, study types included in this review were randomized and non-randomized control trials, pre-post studies without controls, cross sectional studies, repeatability studies and retrospective analyses.

Excluded studies included retrospective analyses of original data already accessible by the authors, case study reports, review articles, and studies with methodology or data presentation which was incomparable with the general body of literature, e.g. studies focusing on single leg exercise in PwMS or studies which failed to provide absolute values for blood lactate concentration.

2.3 Data extraction and quality assessment

Results from database searches were scanned for appropriate headings and abstracts, with eligible papers subsequently imported to a bibliographic database. Duplicates were then removed and whole articles were reviewed in full for inclusion eligibility. All papers which fulfilled criteria for exclusion were removed. The data extracted from the papers included in this review included participant data, intervention protocols and outcomes. Data was extracted by author LC and verified by author NS. Study quality was assessed using the 20-point Appraisal tool for Cross-Sectional Studies (AXIS tool) [22]. The AXIS tool is designed to enable reviewers to incorporate assessment of both bias and study quality into a single score [22],whereby higher scores indicated higher quality of studies with a lower risk of bias and lower scores indicated lower quality of studies with a higher risk of bias.

2.4 Statistical Analysis

Meta-analyses were executed using Comprehensive Meta-Analysis (Biostat, V 2.2.064, Englewood, NJ, USA). A meta-analysis using a random-effects model was used to pool data and compare data between HC and PwMS, and the differences between lactate values before and after training interventions.

Mean, standard deviation and sample size for PwMS and HC for each variable of interest were used to determine overall effect size using a random effects model. Standardised mean differences were utilised in data analysis to accommodate for the variable nature between studies of the timing for lactate testing during exercise bouts and the differences in devices/processes used to measure overall blood lactate concentrations. Due to an outlier with a large sample size in participant number for $Lactate_{REST}$ in PwMS compared to HC [7], a one-study-removed analysis was also performed for this variable.

3.0 Results

3.1. Study selection

The search across the databases previous listed yielded 299 results (Figure 1). 8 papers were additionally identified from hand searching of other sources. In total, 307 papers were screened for exclusion: first by title, then by abstract and finally by full-paper screening before removing duplicates. 23 eligible publications remained, from which 5 were removed: one for being a retrospective analysis of another study already included in systematic review, one for being a case report, one for including ages ranging from 10-50 years without discerning between ages above and below 18 years of age, and two for being incomparable with other studies (Figure 1). The incomparable methods reported in Figure 1 included presentation of only *change* in blood lactate without absolute values, and the use of a unilateral exercise method which measured blood lactate in lower limbs following single-leg exercise. It was for these reasons the studies were deemed incomparable to the other studies involved in the review. For the 18 papers which remained, qualitative analyses were performed with a focus on study quality, MS type EDSS, symptom exacerbations, lactate testing conditions and equipment, and lactate and power output data during rest and sub-maximal and maximal intensity exercise. 15 papers were eligible to be included in at least one meta-analysis of $Lactate_{REST}$ in PwMS compared to HC, $Lactate_{SUB\text{-}MAX}$ in PwMS before and after exercise intervention, and $Lacatate_{MAX}$ in PwMS compared to

196 HC, before and after exercise intervention and between higher and lower scoring EDSS groups. The 3
197 studies which were excluded from meta-analysis, 2 provided data only for $Lactate_{REST}$ in PwMS before
198 and after training intervention (and as such were not numerous enough for meta-analysis) and 1
199 provided incomplete data for the meta-analysis which would otherwise have been appropriate.

200

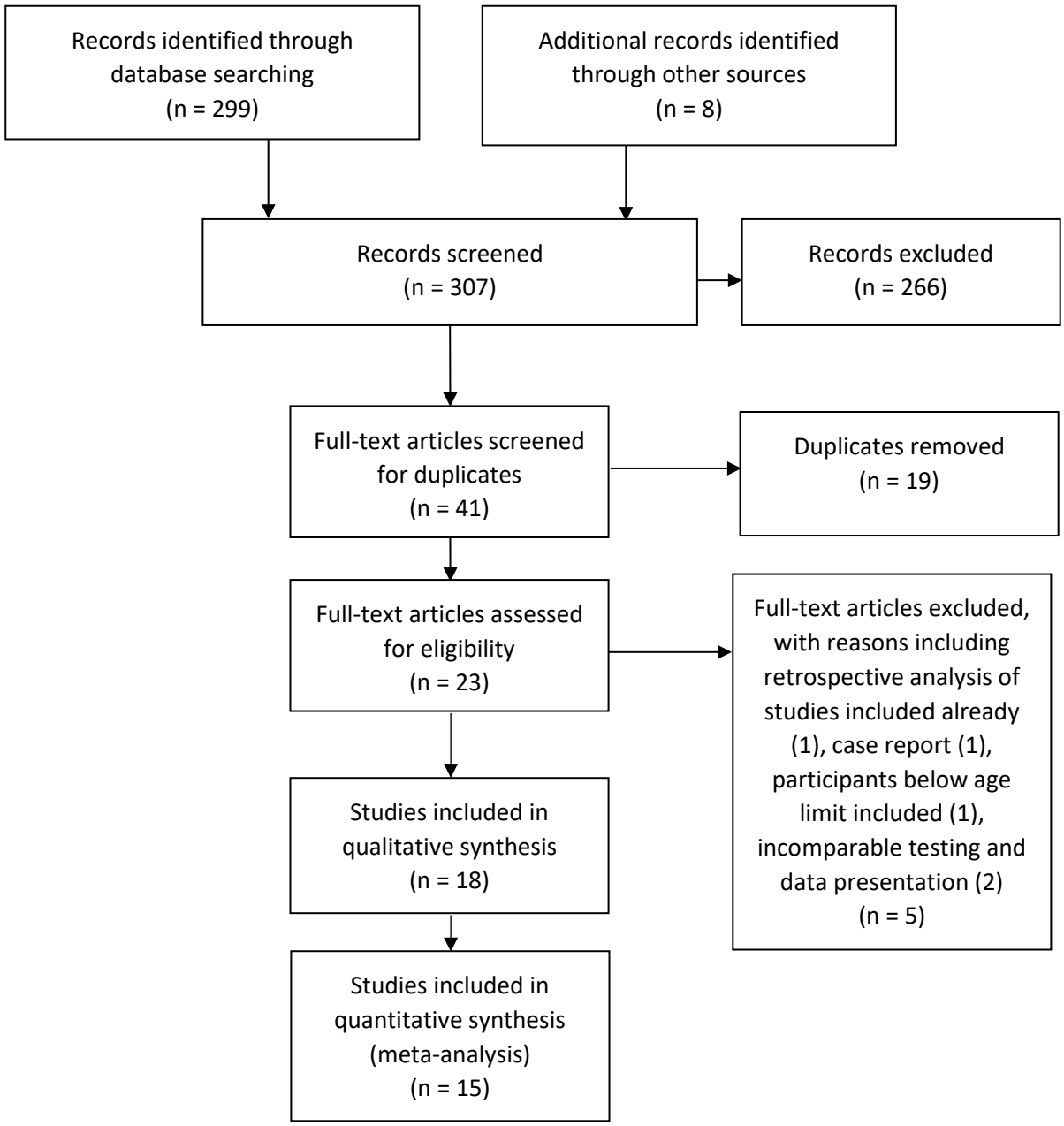


Fig 1: PRISMA flow diagram of literature search and review process

224 Table. 1 – Summary of studies included in systematic review with descriptive statistics and AXIS Score

225

Study	Study design	Final number of participants	Sex f/m	Age (years) ± SD	BMI (kg/m^2) ± SD	EDSS score (mean) ± SD	EDSS score (range)	Maximum EDSS score for study inclusion	MS phenotype (nType)	AXIS Score (x / 20)
Keytsman et al. (2017) [23]	pre-/post no control	n_{MS} = 16	9/7	52.8 ± 7.2	23.5 ± 3.3	2.6 ± 1.5	NR	6	NR	14
Zaenker et al. (2018) [24]	pre-/post no control	n_{MS} = 26 ($n_{EDSS \leq 3}$ = 18 $n_{EDSS\ 3.5\text{-}5}$ = 8)	19/7 (14/4 5/3)	44.6 ± 7.9 (45.6 ± 7.5 42.6 ± 9.6)	24.22 ± 4.7 (24.33 ± 3.61 23.8 ± 6.65)	2.5 ± 1.5 (1.61 ± 0.9 4.38 ± 0.5)	0 – 5 (0 – 3 3.5 – 5)	5	22RR, 3SP, 1PP (17RR, 1PP 5RR, 3SP)	13
Wens et al. (2015) [19]	Randomised control trial	n_{INT} = 29 n_{CTL} = 15	17/12 8/7	48 ± 2 49 ± 2	22.6 ± 4.84 22.9 ± 5.03	3.25 ± 1.08 3.36 ± 1.55	NR	NR	17RR, 12CP 11RR, 4CP	16
Kerling et al. (2015) [18]	Non-controlled	n_{CMBD}= 19 (30) n_{END} = 18 (30)	24/6 20/10	42.3 ± 9 45.6 ± 11.4	24.5 ± 3.6 24.7 ± 4	2.6 ± 1.1 3.1 ± 1.3	NR	6	NR	16

(basic group data provided for pre-drop-out, only)	randomised trial	(pre-drop-out total: n_{MS} = 60)								
Hansen et al. (2015a) [25]	Randomised control trial	n_{INT} = 14 n_{CTL} = 9	10/4 5/4	50 ± 7 46 ± 10	24.8 ± 5.3 24.2 ± 2.6	3.2 ± 1.5 3.1 ± 1.4	NR	NR	9RR, 4SP, 1PP 6RR, 1PR, 2PP	16
Hansen et al. (2015b) [26] 2-part study	Pt 1: Cross-sectional study (MS vs. HC) Pt2: Randomised control trial	Pt 1: n_{MS} = 37 n_{HC} = 15 Pt 2: n_{INT} = 16 n_{CTL} = 11	Pt 1: 22/15 8/7 Pt 2: 10/6 6/5	Pt 1: 48 ± 10 50 ± 10 Pt 2: 46 ± 11 48 ± 10	Pt 1: 25.3 ± 4.7 24.5 ± 2.6 Pt 2: 26.1 ± 5.2 23.4 ± 4	Pt 1: 3.1 ± 1.3 NA Pt 2: 3 ± 1.5 3 ± 1.3	NR	NR	20RR, 10SP, 3PP, 1PR, 3Unknown 12RR, 2SP, 1PP, 1Unknown	16

	(MS_{INT} vs. MS_{CTL})								6RR, 1SP, 2PP, 1PR, 1 Unknown	
Keytsman et al. (2019) [27]	Controlled non-randomised trial	n_{MS} = 18 n_{HC} = 19	6/12 5/14	41.7 ± 8.5 41.5 ± 9.9	24.8 ± 3.9 24.6 ± 2.8	1.9 ± 1.1 NA	NR	4	All phenotypes	17
Amorini et al. (2014) [7]	Cross-sectional	n_{MS} = 613 n_{HC} = 625	411/202 420/205	45.4 ± 12.8 44.8 ± 11.7	NR NR	3.3 ± 2.1 NA	0 - 8	NR	430RR, 153SP, 30PP	18
Cleland et al. (2016) [28]	Repeatability study	n_{MS} = 16 n_{HC} = 11	10/6 6/5	45.7 ± 4.9 42.9 ± 7.5	NR NR	NR NA	1 – 5	NR	9RR, 5CP, 2Unknown	16
Hansen et al. (2013b) [17]	Cross Sectional	n_{MS} = 38 n_{HC} = 16	22/16 9/7	48 ± 10 51 ± 10	25.1 ± 4.7 24.4 ± 2.5	3.1 ± 1.3 N/A	NR	NR	20RR, 14SP, 3PP, 1PR	14

Hansen et al. (2013a) [29]	Cross sectional	n_{MS} = 26 n_{HC} = 15	16/10 9/6	50 ± 10 50 ± 10	25 ± 3.5 24.2 ± 2.5	3.4 ± 1.3 NA	NR	NR	14RR, 9SP, 3PP	14
Juybari et al. (2018) [30]	Cross sectional	n_{MS} = 50 n_{HC} = 50	35/15 37/13	49.6 ± 13.5 45.3 ± 15.3	26.1 ± 4.9 25.1 ± 4.9	3.8 ± - NA	NR	NR	50RR	13
Kerling et al. (2014) [31]	Cross sectional	n_{MS} = 60 ($n_{EDSS<3}$ = 38 $n_{EDSS\ 3.5\text{-}6}$ = 22) n_{HC} = 48	44/16 (28/10 16/6) 36/12	44 ± 10 (42.6 ± 10.5 46.6 ± 9.9) 40.0 ± 13.7	24.6 ± 3.8 (25.2 ± 4.3 23.6 ± 2.4) 25.2 ± 4.6	NR NR NR NA	0 – 6 (0 – 3 3.5 – 6)	6	NR	18
Langeskov-Christensen et al. (2014) [32]	Repeatability study	n_{MS} = 20 n_{HC} = 20	11/9 11/9	39.9 ± 9 40 ± 8	24.6 ± 4.8 24.1 ± 2.7	2.6 ± 1.6 NA	NR	6	19RR, 1SP	17

Larson et al. (2014) [33]	Cross sectional	n_{MS} = 8 n_{HC} = 7	6/2 5/2	51.1 ± 9.2 49.4 ± 14.3	25 ± 3.9 26.0 ± 8.3	NR NA	0 – 5	6.5	8RR	13
Penesova et al. (2015) [34]	Cross sectional	n_{MS} = 19 n_{HC} = 19	10/9 10/9	30 ± 7 29 ± 7	23.8 ± 4.5 24.4 ± 5.3	1.1 ± 0.8 NA	NR	2	NR	16
Schlüter et al. (2017) [35]	Retrospective analysis	n_{RR} = 25 n_{CP} = 41	16/9 23/18	40 ± 11 50 ± 8	24.1 ± 5.5 23.1 ± 3.3	NR NR	0 – 3.5 4 – 6	6	25RR 14CP	15
't Eijnde et al. (2014) [36]	Cross sectional	n_{MS} = 12 n_{HC} = 12	8/4 7/5	54 ± 7 50 ± 9	24.8 ± 3.9 24.7 ± 3.3	3.5 ± 1.5 NA	NR	NR	6RR, 2SP, 1PP	16

Abbreviations: Healthy Controls (HC), Multiple Sclerosis (MS), number (n_x), intervention (INT), control (CTL), combined training (CMBD), endurance training (END), relapsing remitting (RR), secondary progressive (SP), primary progressive (PP), chronic progressive (CP), progressive relapsing (PR), not reported (NR), not applicable (NA), standard deviation (SD).

228 3.2 Study quality

229

230 With 1 exceptional outlier with a sample size totalling 1238 participants (613 with MS) [7], 6 studies
231 had less than 30 participants [23–25,28,33,36], 9 studies had between 30 and 70 participants [17–
232 19,26,27,29,32,34,35] and 2 studies had between 100-110 participants [30,31]. The total number of
233 participants reported on across all studies was 1986; 1129 were participants with MS. 5 papers failed
234 to adequately describe their methods adequately for study reproduction [23,24,30,31,33]. Of the
235 studies conducting intervention trials, 3 did not include a control group [18,23,24]. 1 study failed to
236 report lactate measurement data for all groups included within the study [32]. Overall AXIS scores may
237 be found in Table 1. The most common sources of points being deducted (and implicit increase in risk
238 of bias) included lack of information on or categorisation of non-responders [17–19,23,25,26,28–
239 31,33–36], lack of justification of sample size used within the study [24,29,30,33–35], potential
240 inherent bias within or lack of description of the population from which samples were recruited
241 [17,19,23–26,29,33,36], and inadequate descriptions of methodologies used within the studies to
242 enable repetition in future research [23,24,28,30,33].

243

244 3.3 MS type

245

246 5 studies [18,23,27,31,34] did not specify which or how many phenotypes of MS they included in their
247 studies. Of the of the remainder, all included a majority of participants with RR MS, 7 studies involved
248 participants with PP MS [7,17,24–26,29,36], 8 studies involved participants with SP MS [7,17,24–
249 26,29,32,36], 3 studies involved participants with CP MS [19,28,35], 3 studies involved 1 participant
250 with PR MS [17,25,26] and 2 studies involved participants with an undisclosed MS phenotype [26,28].
251 MS phenotypes included in each study are located in Table 1.

252

253 3.4 EDSS

All studies provided a description of the EDSS status of participants in the form of a group mean EDSS value, group EDSS range, and/or maximal EDSS value eligible for inclusion in the study.

12 studies reported a mean EDSS value between 2 – 4.5 [7,17–19,23–26,29,30,32,36], 2 studies reported a mean EDSS value less than 2 [27,34] and 4 studies did not report mean EDSS value [28,31,33,35].

1 study [34], focusing on people newly diagnosed with MS, limited EDSS value to ≤2 to be included in their study, 9 studies were limited to a maximum EDSS score of ≤6 or included an EDSS score range within 0-6 [18,23,24,27,28,31–33,35], 1 study included an EDSS range between 0-8 [7] and 7 studies reported neither range nor maximal inclusion values for EDSS [17,19,25,26,29,30,36]. 3 studies separated EDSS into lower and higher range brackets for direct comparison of blood lactate concentration between disease severities for PwMS, with the minimum score for higher EDSS grouping placed at 3.5 or 4 [24,31,35].

No studies involving exercise interventions ≥3 months reported reassessed EDSS scores after completion of the intervention. However, 1 study [18] stated in their discussion that EDSS remained stable in participants with MS following a 3 month exercise intervention.

Mean EDSS scores, EDSS score range, and maximum EDSS scores included in each study are located in Table 1.

3.5 Symptom exacerbation

279 4 studies [18,19,24,27] reported instances of participants with MS dropping out of an intervention as
280 a result of symptom exacerbation or relapse. No studies reported on frequency of symptom
281 exacerbation or relapse as a measure of intervention effectiveness.
282
283 3.6 Testing Conditions
284
285 7 papers tested for blood lactate under resting conditions [7,19,25,28,30,34,36], 7 tested during
286 submaximal exercise [17–19,25,26,29,36] and 8 papers tested during maximal intensity exercise
287 [18,23,24,27,31–33,35].
288
289 All 14 studies which involved lactate testing during exercise used a cycle ergometer to reach the
290 desired exercise intensity, however exercise protocol differed between studies. Details of testing
291 conditions can be found in Table 3 for intervention studies and Table 4 for observational studies.
292
293 3.7 Lactate testing equipment
294
295 1 study [33] did not describe their methods of lactate measurement but reported the study as part of
296 another [37] which did provide a detailed description but could not itself be included in group
297 comparison. 7 papers measured blood lactate using the Accutrend Plus lactate analyser [17,19,25–
298 27,29,36], 2 papers with the EBIO 6666 lactate analyser [18,31], 2 reported using the Lactate Pro
299 [28,33], 1 used the BioSen C-Line [35], 1 used the YSI 1500 Sport [32], 1 used the SUPER GL2 [34], 1
300 used an unbranded colorimetric assay kit [30], 1 used spectrophotometric determination [7], and 2
301 did not describe their methods of determining blood lactate [23,24]; those two who did not describe
302 their method of lactate measurement were testing blood lactate concentration during maximal
303 intensity exercise. Lactate testing equipment details are located in Table 3 for intervention studies and
304 Table 4 for observational studies.

305

306 3.8 Lactate testing sites

307

308 9 studies took blood from the fingertip [17,19,25,26,28,29,32,33,36], 3 from the earlobe [18,27,35], 2
309 from the cubital vein [7,34], and 4 provided no description of the testing site [23,24,30,31].

310

311 3.9 Timings and Workloads of Lactate Testing

312

313 Full details of individual studies' lactate measurement timing are provided in Table 3 for intervention
314 studies and Table 4 for observational studies.

315

316 3.9.1 $Lactate_{REST}$

317

318 2 studies measured resting blood lactate in participants after 15 minutes rest [7,36], with 1 study [36]
319 sitting participants on the cycle ergometer before testing blood lactate, 3 studies measured resting
320 blood lactate with participants on the cycle ergometer within 3 minutes or immediately prior to
321 beginning a bout of exercise [19,25,28], 1 study tested their participants in an early morning fasted
322 state but did not rest participants before blood sampling [34], and 1 study provided no description of
323 resting protocol for participants prior to blood sampling [30].

324

325 3.9.2 $Lactate_{SUB\text{-}MAX}$

326

327 4 studies measured blood lactate in participants at the end of submaximal intensity exercise bouts
328 [17,19,25,29], 2 studies measured blood lactate in participants during submaximal exercise bouts
329 [26,36], and 1 study measured blood lactate at a 50W workload during increments of a

cardiopulmonary exercise test (CPET) [18]. Details of relative or absolute submaximal exercise intensities are located in Table 3.

3.9.3 $Lactate_{MAX}$

4 studies measured blood lactate at the end of a CPET [24,32], 2 studies measured blood lactate concentration both at the end of a CPET ($Lactate_{MAX}$) and the highest value obtained during the recovery phase following a CPET ($Lactate_{PEAK}$) [23,27], 2 studies measured blood lactate 1 minute into and every subsequent 3 minutes during a CPET [18,31], 1 study measured blood lactate 3 minutes after cessation of a CPET [33], and 1 study made multiple blood lactate tests during the recovery phase post-CPET and reported the highest as $Lactate_{MAX}$ [35].

3.10 Power output during lactate testing

3.10.1 Maximum power output in PwMS compared with HC

3 studies reported maximum power output (W) reported during CPETs in PwMS compared with HC [27,31,33]. All studies reported lower absolute (W) mean power output at maximum exercise intensity in PwMS compared with HC, however only 1 study [31] calculated this difference to be statistically significant. 1 study [27] did not statistically compare power output between PwMS and HC and another [33] reported a statistically insignificant difference between PwMS and HC group means.

3.10.2 Maximum power output in PwMS with low EDSS compared with high EDSS scores

4 studies reported maximum power output reported during CPETs in PwMS comparing groups with low and high EDSS scores [24,31,35]. One study [31] reported significantly higher mean absolute (W)

and relative (W/kg) power output at maximum intensity exercise in the low EDSS group compared with the high EDSS group. One study [35] reported significantly higher mean relative power output at maximum intensity exercise in the low EDSS group compared with the high EDSS group. One study [24] reported higher mean power output at maximum intensity exercise in the low EDSS group compared with the high EDSS group but did not statistically analyse the significance of this difference.

3.10.3 Sub-maximal power output in PwMS pre-intervention compared with post-intervention

Of the 3 studies investigating blood lactate in PwMS during sub-maximal intensity exercise before and after a training intervention [19,25,26], all studies calculated target power output for each participant using calculations based on age, sex, height and body mass. 2 studies used the same absolute (W) power output during the sub-maximal exercise test before and after the training intervention [25,26] and 1 study [19] did not specify if the estimated power output was recalculated at baseline and post-intervention. Intervention exercise prescription details are located in Table 2.

3.10.4 Maximum power output in PwMS pre-intervention compared with post-intervention

4 studies reported absolute power output (W) at maximum intensity during a CPET in PwMS before and after a training intervention [23,24,27,31]. 3 studies reported significantly increased power output in PwMS post-intervention compared to baseline [23,24,27]. 1 study reported a small increase in mean power output in both groups of PwMS (combined exercise group and endurance exercise group) following an exercise intervention but did not provide statistical analysis of these changes[31]. Intervention exercise prescription details are located in Table 2.

3.11 Oxygen Consumption

382 3.11.1 Oxygen consumption at rest ($VO2_{REST}$) in PwMS compared to HC

383 3 studies reported $VO2_{REST}$ in PwMS and HC [17,28,36], however only 1 of these provided statistical
384 comparison of these values [17]. Similar or statistically non-significant differences in $VO2_{REST}$ were
385 reported in 2 papers [17,28], while 1 paper [36] reported a modestly higher $VO2_{REST}$ in HC compared
386 to MS, though confirmation of the statistical significance of this was not provided.

387 3.11.2 Oxygen consumption at maximal intensity exercise ($VO2_{MAX}$) in PwMS compared to HC

388 4 studies reported oxygen consumption during maximal intensity ($VO2_{MAX}$) exercise in PwMS and HC
389 [31–33,38]. Significantly greater $VO2_{MAX}$ values were reported in HC compared to PwMS in 3 studies
390 [31–33], meanwhile 1 study [38] reported higher $VO2_{MAX}$ values for HC compared to PwMS but did
391 not report the statistical significance of this difference.

392 3.11.3 Oxygen consumption at sub-maximal intensity exercise ($VO2_{SUB\text{-}MAX}$) in PwMS pre- vs. post-
393 intervention

394 2 studies [25,26] reported $VO2_{SUB\text{-}MAX}$ values for PwMS pre- compared to post-exercise intervention,
395 neither study reported significant differences in $VO2_{SUB\text{-}MAX}$ following the exercise interventions
396 provided to their cohorts. Intervention exercise prescription details are located in Table 3.

397 3.11.4 $VO2_{MAX}$ in PwMS pre- vs. post-intervention

398 3 studies [18,24,38] reported $VO2_{MAX}$ values in PwMS before and after completing an exercise
399 intervention. 2 studies [24,38] reported significant increases in $VO2_{MAX}$ in their cohort of PwMS
400 following an exercise intervention, meanwhile 1 study [18] reported non-significant increases in
401 mean $VO2_{MAX}$ in their cohort of PwMS following either a combined-exercise or endurance-exercise
402 intervention. Intervention exercise prescription details are located in Table 3.

403

404 Table 2. Summary of exercise intervention studies

Study	Study design	Lactate testing state	Intervention type -Supervision level	Intervention progression	Frequency (sessions/2wk)	Intervention duration (months)
Keytsman et al. (2017) [23]	pre-/post no control	Maximal intensity exercise	Resistance training and HIIT - Supervised	Wk 1-6: Cycling HIIT sessions gradually increased from 5x1min bouts > 5x2min bouts at 85-90% HR_{MAX}. Wk 7-12: 5x2min bouts at 100%HR_{MAX} and resistance training for upper and lower body altering within range of 1x10 and 2x20 repetitions, depending on individual ability.	5	3
Zaenker et al. (2018) [24]	pre-/post no control	Maximal intensity exercise	Resistance training, endurance training and HIIT	Wk1-4: 1 endurance session (intervals and continuous blend) and 1 resistance training session for lower body per week, progressing from 4x10 > 5x15 repetitions. 15-20mins stretching at end of sessions.	1^{st} month: 4	3

			-Supervised and autonomous	Wk 5-12: One additional autonomous session added, alternating between endurance and resistance each week.	2^{nd} + 3^{rd} months: 5	
Wens et al. (2015) [19]	Randomised control trial	Rest, Submaximal intensity exercise	Cardiovascular and resistance training -Supervised	Control group: no intervention Intervention group: Sessions began with cardiovascular training (walk, run or cycle) followed by upper and lower body resistance training. Cardiovascular training ranged from 1x6min > 3x10min bouts, and resistance training ranged from 1x10 > 4x15 repetitions. Volume and intensity of CV and resistance gradually increasing through the programme.	5	6
Kerling et al. (2015) [18]	Non-controlled	Submaximal, maximal intensity exercise	MS_{CMBD}: Endurance and resistance training	MS_{CMBD}+MS_{END}: 1^{st} phase began with 20 minutes of endurance training on cycle ergometer at 50% $workload_{MAX}$, Borg≤13.	4	3

	randomized trial		MS_{END}: Endurance training only -Supervised	MS_{END}: 2nd phase of endurance training continued for further 20minutes on X-trainer, stepper, arm ergometer, treadmill or rowing ergometer, as chosen by participant. HR kept within parameters determined by the exercise method chosen. MS_{CMBD}: 2nd phase was a full-body dynamic resistance training programme. Participants aimed for 2x10-15 repetitions for each exercise, intensity was increased once 2x15repetitions were achieved.		
Hansen et al. (2015a) [25]	Randomised control trial	Rest, submaximal intensity exercise	Endurance and resistance training -Supervised	Control group: no intervention Intervention group: Sessions began with cycling, walking or running endurance training, gradually increasing from 1x6 > 3x10 minutes and corresponding to 12-14 Borg scale rating.	5	6

				This was followed by upper and lower body resistance training, gradually increasing from 1x10 > 4x15 repetitions as self-determined by participant with encouragement from supervisor. Intensity corresponded to 12-14 Borg scale rating.		
Hansen et al. (2015b) [26] 2-part study	Pt 1: Cross-sectional study (MS vs. HC) Pt2: Randomised control trial (MS_{INT} vs. MS_{CTL})	Submaximal intensity exercise	Endurance and resistance training -Supervised	For Pt2 of study: Control group: no intervention Intervention group: Sessions began with cycling or walking endurance training, gradually increasing from 1x6 > 3x10 minutes and corresponding to 12-14 Borg scale rating. This was followed by upper and lower body resistance training, gradually increasing from 1x10 > 4x15 repetitions as self-determined by participant with encouragement from supervisor.	5	6

				Intensity corresponded to 12-14 Borg scale rating.		
Keytsman et al. (2019) [27]	Controlled non-randomised trial	Maximal intensity exercise	Periodized endurance and HIIT -Home-based or group	Training programme progressed through 8 3-week cycles: Wk1 – High intensity endurance training Wk2 – Max intensity interval training Wk3 – Interval training and optional endurance sessions Session structures ranged from 3x60-90 second intervals at 90-100% HR_{MAX}, 1-3 hour sessions at 60-80% HR_{MAX}, or 40-55km at 60-80% or 70-90% HR_{MAX}. All sessions were undertaken on cycle ergometers.	7-8/3weeks	6

405 Abbreviations: High intensity interval training (HIIT), combined exercise (CMBD), endurance exercise (END), heart rate (HR)

406

407

408 Table 3. Blood Lactate conditions and outcomes in exercise intervention studies

Study	Lactate testing state	Test exercise equipment	Exercise test type	Lactate testing apparatus	When was blood lactate measured?	Pre-Intervention Lactate concentration (mmol/L) ± SD	Post-Intervention Lactate concentration (mmol/L) ± SD
Keytsman et al. (2017) [23]	Maximal intensity exercise	Cycle ergometer: eBike BasicVR, General Electric GmbH, Bitz, Germany	CPET	No description given	Not reported, but identical result format as Keytsman et al. (2019): $Lactate_{MAX}$ taken at cessation of CPET and $Lactate_{PEAK}$ was greatest value recorded during recovery	$Lactate_{MAX}$: MS – 5.8 ± 1.8 $Lactate_{PEAK}$: MS – 8.2 ± 2.8	$Lactate_{MAX}$: MS – 5.7 ± 1.4 $Lactate_{PEAK}$: MS – 10.3 ± 2.2

Zaenker et al. (2018) [24]	Maximal intensity exercise	Cycle ergometer: Ergoline type Ergoselect 200P	CPET	No description given	End of test	$Lactate_{MAX}$: MS_{ALL} – 6.9 ± 2.2 $MS_{EDSS\ 0\text{-}3}$ – 7.4 ± 1.6 $MS_{EDSS\ 3.5\text{-}5}$ – 5.9 ± 2.8	$Lactate_{MAX}$: MS_{ALL} – 9.1 ± 3.1 $MS_{EDSS\ 0\text{-}3}$ – 9.3 ± 2.2 $MS_{EDSS\ 3.5\text{-}5}$ – 8.5 ± 4.4
Wens et al. (2015) [19]	Rest, Submaximal intensity exercise	Cycle ergometer: eBike Basic; General Electric GmbH, Bitz, Germany	25% W_{MAX}	Accutrend Plus	3-minute resting period before exercise and end of exercise bouts 1 (B1) and 2 (B2).	$Lactate_{REST}$: MS_{INT} – 2.6 ± 0.54 MS_{CTL} – 2.6 ± 0.77 $Lactate_{SUB\text{-}MAX}$: MS_{INT} – (B1) 3 ± 0.54 (B2) 2.8 ± 1.08 MS_{CTL} – (B1) 3.1 ± 0.77 (B2) 3.1 ± 0.77	$Lactate_{REST}$: MS_{INT} – 2.2 ± 0.54 MS_{CTL} – 2.3 ± 0.77 $Lactate_{SUB\text{-}MAX}$: MS_{INT} – (B1) 2.6 ± 0.54 (B2) 2.5 ± 0.54 MS_{CTL} – (B1) 3.3 ± 1.16 (B2) 3.1 ± 0.77
Kerling et al. (2015) [18]	Submaximal, maximal intensity exercise	Cycle ergometer: Ergometrics 900s, Ergoline, Bitz, Germany	CPET	EBIO 6666	1 minute after beginning of CPET, every 3 minutes during exercise,	$Lactate_{50W}$: MS_{CMBD} – 1.45 ± 0.51 MS_{END} – 1.57 ± 0.79 $Lactate_{MAX}$:	$Lactate_{50W}$: MS_{CMBD} – 1.31 ± 0.51 MS_{END} – 1.35 ± 0.52 $Lactate_{MAX}$:

					and at 50W in CPET	MS_{CMBD} – 5.43 ± 2.03 MS_{END} – 4.8 ± 2.89	MS_{CMBD} – 5.9 ± 1.97 MS_{END} – 5.14 ± 2.5
Hansen et al. (2015a) [25]	Rest, submaximal intensity exercise	Cycle ergometer: eBike Basic, General Electric GmbH, Bitz, Germany	25% W_{MAX}	Accutrend Plus	3 minute resting period before exercise and end of exercise bout	$Lactate_{REST}$: MS_{INT} – 2.7 ± 0.4 MS_{CTL} – 2.4 ± 0.7 $Lactate_{SUB-MAX}$: MS_{INT} – 3.1 ± 0.9 MS_{CTL} – 3.1 ± 0.5	$Lactate_{REST}$: MS_{INT} – 2 ± 0.6 MS_{CTL} – 2.2 ± 0.6 $Lactate_{SUB-MAX}$: MS_{INT} – 2.4 ± 0.7 MS_{CTL} – 3.3 ± 1.1
Hansen et al. (2015b) [26]	Submaximal intensity exercise	Cycle ergometer: eBike Basic, General Electric GmbH, Bitz, Germany	MS – 25% W_{MAX} HC – 35% W_{MAX}	Accutrend Plus	During final minute of exercise	$Lactate_{SUB-MAX}$: Pt 1: MS_{ALL} – 3.1 ± 0.8 HC – 3.1 ± 1.1 Pt 2: MS_{INT} – 3.2 ± 0.8 MS_{CTL} – 3.4 ± 0.6	$Lactate_{SUB-MAX}$: Pt 1: N/A Pt 2: MS_{INT} – 2.5 ± 0.7 MS_{CTL} – 3.6 ± 1

Keytsman et al. (2019) [27]	Maximal intensity exercise	Cycle ergometer: eBike Basic, General Electric GmbH, Bitz, Germany	CPET	Accutrend Plus	$Lactate_{MAX}$ taken at cessation of CPET and $Lactate_{PEAK}$ was greatest value recorded during post-exercise recovery	$Lactate_{MAX}$: MS – 4.5 ± 1.6 HC – 5.3 ± 1.3 $Lactate_{PEAK}$: MS – 8.9 ± 2.3 HC – 9.7 ± 2.4	$Lactate_{MAX}$: MS – 5.9 ± 1.8 HC – 6.9 ± 2.1 $Lactate_{PEAK}$: MS – 10.3 ± 3.1 HC – 10.7 ± 2.2

Abbreviations: not applicable (N/A), not stated (N/S), Multiple Sclerosis group (MS), healthy control group (HC), control group (CTL), intervention group (INT), combined-exercise group (CMBD), endurance-only group (END), cardiopulmonary exercise test (CPET), maximal workload (W_{MAX}), standard deviation (SD), Lactate value taken at cessation of CPET ($Lactate_{MAX}$), greatest lactate value recorded during post-exercise recovery ($Lactate_{PEAK}$).

414 Table 4. Blood Lactate conditions and outcomes in observational studies

Study	Lactate testing state	Test exercise equipment	Exercise test type	Lactate testing apparatus	When was blood lactate measured?	Lactate concentration (mmol/L) ± SD	
Amorini et al. (2014) [7]	Rest	N/A	N/A	Spectrophotometric determination	Following 15 minutes of complete rest	$Lactate_{REST}$: MS - 3.04 ± 1.26 HC - 1.09 ± 0.25	
Cleland et al. (2016) [28]	Rest	N/A	N/A	Lactate Pro	Seated on cycle ergometer before exercise	$Lactate_{REST}$: Week 1: MS - 2.9 ± 2.6 HC - 2.1 ± 0.8	$Lactate_{REST}$ Week 2: MS - 1.9 ± 0.5 HC - 2.2 ± 1.1
Hansen et al. (2013b) [17]	Submaximal intensity exercise	Cycle ergometer: eBike Basic, General Electric GmbH, Bitz, Germany	MS – 25% W_{MAX} HC – 35% W_{MAX}	Accutrend Plus	End of exercise bouts 1 (B1) and 2 (B2).	$Lactate_{SUB\text{-}MAX}$: MS – (B1) 3.1 ± 0.8 (B2) 3.0 ± 0.7 HC – (B1) 3 ± 1.1 (B2) 2.6 ± 1.4	

Hansen et al. (2013a) [29]	Submaximal intensity exercise	Cycle ergometer: eBike Basic, General Electric GmbH, Bitz, Germany	MS – 25% W_{MAX} HC – 35% W_{MAX}	Accutrend Plus	End of exercise bout.	$Lactate_{SUB-MAX}$: MS - 2.9 ± 0.6 HC - 3.1 ± 1.1
Juybari et al. (2018) [30]	Rest	N/A	N/A	Lactate assay kit	N/S	$Lactate_{REST}$: MS - 2.5 ± 0.6 HC - 1.6 ± 0.34
Kerling et al. (2014) [31]	Maximal intensity exercise	Cycle ergometer: Ergoline viasprint 150 P, ergoline, Bitz, Germany	CPET	EBIO 6666	1 minute after beginning of CPET and every subsequent 3 minutes.	$Lactate_{MAX}$: MS_{ALL} - 5.09 ± 2.52 HC - 9.17 ± 2.9 $MS_{EDSS\ 0-3}$ - 5.86 ± 2.5 $MS_{EDSS\ 3.5-6}$ - 3.77 ± 2.01

Langeskov-Christensen et al. (2014) [32]	Maximal intensity exercise	Cycle ergometer: Monark Ergomedic 828E, Monark AB, Sweden	CPET	YSI 1500 Sport	Within 1 minute after cessation of CPET.	$Lactate_{MAX}$: Test 1: MS - 9.5 ± 2.7 Test 2: MS - 9.4 ± 2.6
Larson et al. (2014) [33]	Maximal intensity exercise	Cycle ergometer: Lode; Groningen, the Netherlands	CPET	No description given	3 minutes after CPET [37]	$Lactate_{MAX}$: MS - 5.7 ± 2.5 HC - 6.7 ± 1.8
Penesova et al. (2015) [34]	Rest	N/A	N/A	SUPER GL2	8AM fasted state	$Lactate_{REST}$: MS - 1.16 ± 0.38 HC - 1.00 ± 0.22
Schlüter et al. (2017) [35]	Maximal intensity exercise	Cycle ergometer: Ergofit® 3000	CPET	Biosen C-Line	Multiple blood tests taken during recovery phase following CPET,	$Lactate_{MAX}$: $MS_{EDSS\ 0\text{-}3.5}$ - 6.5 ± 2.4 $MS_{EDSS\ 4\text{-}6}$ - 4.1 ± 2

					with the highest value recorded as $Lactate_{MAX}$.		
't Eijnde et al. (2014) [36]	Rest, submaximal intensity exercise	Cycle ergometer: eBike Basic, General Electric GmbH, Germany	MS – 25% W_{MAX} HC – 35% W_{MAX}	Accutrend Plus	During 3 minutes of rest on bike after 15 minutes of seated rest in laboratory and during exercise bout 1 (B1) and 2 (B2).	Control (Week 1): $Lactate_{REST}$: MS - 2.9 ± 0.8 HC - 2.5 ± 0.8 $Lactate_{SUB-MAX}$: MS – (B1) 2.7 ± 0.9 (B2) 2.7 ± 0.9 HC – (B1) 3 ± 1.1 (B2) 2.3 ± 0.7	Precooling (Week 2): $Lactate_{REST}$: MS – 3.2 ± 0.9 HC – 3.1 ± 1.1 $Lactate_{SUB-MAX}$: MS – (B1) 3.3 ± 0.9 (B2) 2.9 ± 0.8 HC – (B1) 3.2 ± 0.9 (B2) 2.9 ± 1.2

415 Abbreviations: not applicable (N/A), not stated (N/S), Multiple Sclerosis (MS), healthy controls (HC), relapsing remitting (RR), chronic progressive (CP), cardiopulmonary exercise test (CPET),

416 maximal workload (W_{MAX}), standard deviation (SD).

417 3.12 Meta Analyses

418

419 15 studies in total were included for 5 meta-analysis comparisons: resting lactate concentrations
420 between HC and PwMS [7,28,30,34,36]; maximum lactate concentrations between HC and PwMS
421 [27,31,33]; maximum lactate concentration between higher EDSS scores and lower EDSS scores in
422 PwMS [24,31,35]; submaximal lactate concentrations pre and post training in PwMS ([19,25,26]; and
423 maximum lactate concentrations pre and post training in PwMS [18,23,24,27]. The descriptive
424 statistics of these meta-analyses are located in Table 5. 4 desirable meta-analyses were not possible
425 to perform due to limited or no data ($Lactate_{SUB\text{-}MAX}$ in HC vs. MS, $Lactate_{REST}$ in High vs. Low EDSS,
426 $Lactate_{SUB\text{-}MAX}$ in High vs. Low EDSS, $Lactate_{REST}$ in PwMS Pre- vs. Post-intervention).

427

428

429 Table 5: Summary statistics of relationships between the studies included in each meta-analysis,
430 illustrated in Figures 2-6.

	HC vs. MS $Lactate_{REST}$ Figure 2.	HC vs. MS $Lactate_{MAX}$ Figure 3.	High vs. Low EDSS $Lactate_{MAX}$ Figure 4.	Pre vs. Post $Lactate_{SUB-MAX}$ Figure 5.	Pre vs. Post $Lactate_{MAX}$ Figure 6.
Q-value	34.72	7.71	0.617	0.237	8.089
DF	4	2	2	2	4
P-Value	<0.001	0.021	0.735	0.88	0.088
I^2 value	88.48	74.081	0.000	0.000	50.54

431 Abbreviations: Degrees of Freedom (DF).

432

433 3.12.1 Resting Lactate

434 Compared to HC, blood lactate concentration at rest was significantly higher in PwMS ($p<0.001$ with
435 a standardised difference in means of 1.43 (95% CI [0.788, 2.094]). From the summary statistics (Table
436 5), the I^2 value of 88.48 and p-value of <0.001 illustrate a statistically significant, substantial
437 heterogeneity between outcomes of the studies included in the meta-analysis. The direction of the
438 effect was the same across all studies, though the effect was not significant in 2 of the studies included
439 within the meta-analysis [28,36]. Significant elevation in the pooled resting lactate in PwMS remained
440 with a standardised difference in means of 1.18 when the results of Amorini et al. (2014) were
441 removed from the meta-analysis.

442

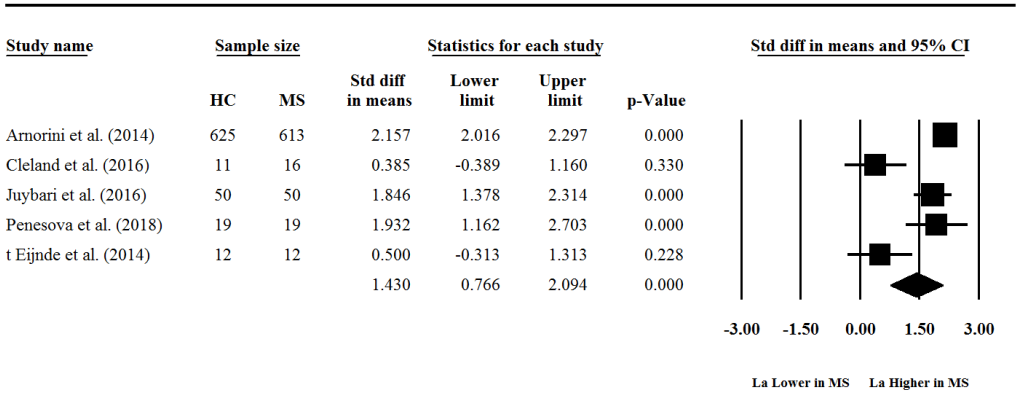


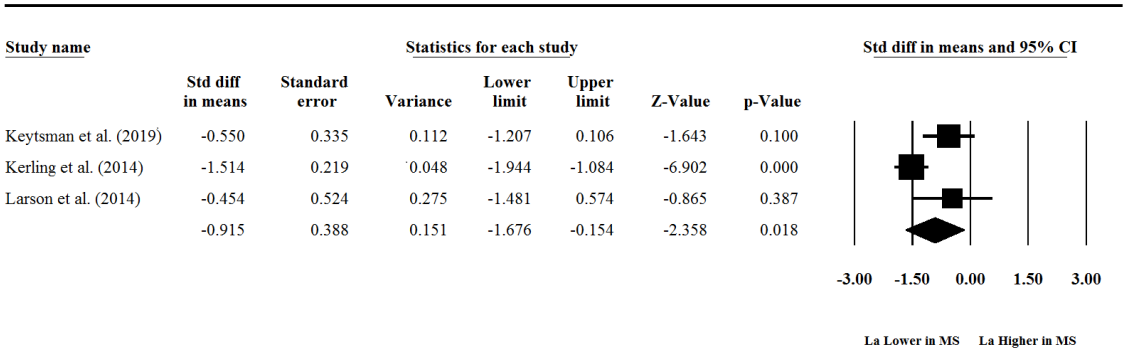
Fig. 2 Forest plot of resting lactate concentrations obtained from PwMS compared with HC, with the final row representing results of data analysis synthesis. Abbreviations: Lactate (La).

448 3.12.2 Maximal Lactate

449

450 Compared to HC, blood lactate concentration in response to maximal intensity exercise was
451 significantly lower in PwMS ($p<0.001$) with a standardised difference in means of 1.141 (95% CI [0.801,
452 1.480]). From the summary statistics (Table 5), the I^2 value of 74.081 and p-value of 0.021 illustrate a
453 statistically significant, moderate to substantial heterogeneity between outcomes of the studies
454 included in the meta-analysis. The same direction of effect was observed across all studies, however,
455 only the results of Kerling et al (2014) were statistically significant, despite a significant pooled effect
456 across all studies.

457



458

459 **Fig. 3** Forest plot of maximal lactate concentrations obtained from PwMS compared with HC, with the

460 final row representing results of data analysis synthesis. Abbreviations: Lactate (La)

461

462

463 3.12.3 Maximal Lactate, High EDSS vs. Low EDSS

464

465 Compared to higher EDSS score counterparts, blood lactate concentration in response to maximal
466 intensity exercise was significantly higher in PwMS with lower EDSS scores ($p<0.001$) with a
467 standardised difference in means of 0.963 (95% CI [0.614, 1.312]). From the summary statistics (Table
468 5), the I^2 value of 0 and p-value of 0.735 illustrate no evidence of significant heterogeneity between
469 outcomes of the studies included in the meta-analysis with a similar direction and magnitude of effect
470 within all studies, despite the differences reported within the study by Zaenker et al. (2018) being non-
471 significant.

472

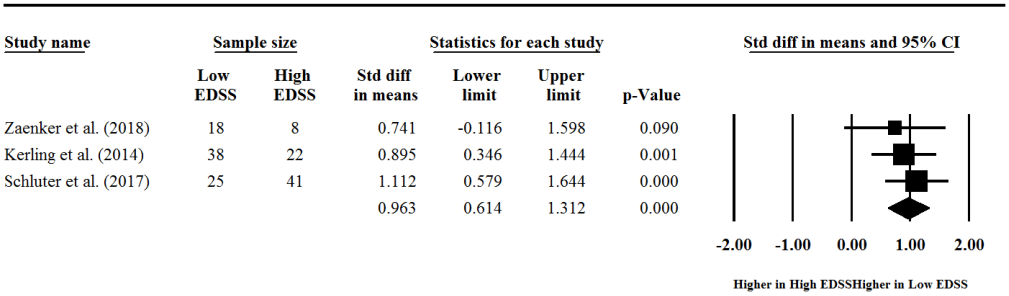
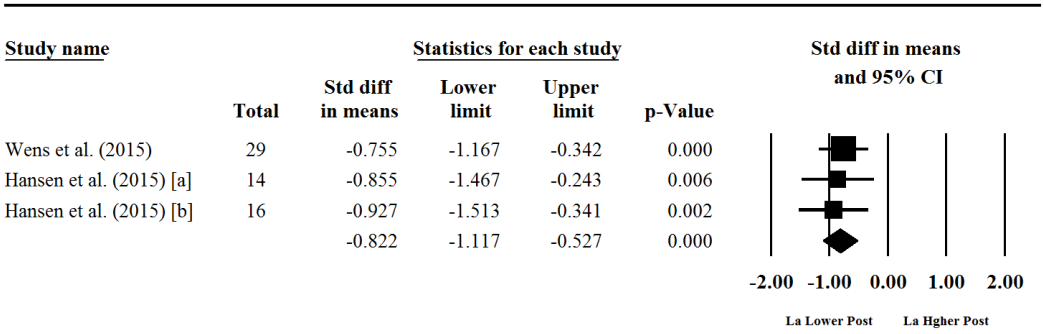


Fig. 4 Forest plot of maximal lactate concentrations obtained in studies which compared groups of higher EDSS scoring individuals compared with maximal lactate concentrations obtained in groups of lower EDSS scoring individuals, with the final row representing results of data analysis synthesis.

3.12.4 Sub-maximal Lactate, Pre-/Post-Intervention

Compared to pre-intervention values, blood lactate concentration in response to sub-maximal intensity exercise was significantly lower post-intervention in PwMS ($p<0.001$) with a standardised difference in means of -0.822 (95% CI [-1.117, -0.527]). From the summary statistics (Table 5), the I^2 value of 0 and p-value of 0.88 illustrate no evidence of significant heterogeneity between outcomes of the studies included in the meta-analysis with a similar direction and magnitude of effect within all studies.



488

489 **Fig. 5** Forest plot of sub-maximal lactate concentrations obtained in PwMS before and after

490 completion of exercise intervention, with the final row representing results of data analysis synthesis.

491 Abbreviations: Lactate (La)

492

493

3.12.5 Maximal Lactate, Pre-/Post-Intervention

Compared to pre-intervention values, blood lactate concentration in response to maximal intensity exercise was significantly higher post-intervention in PwMS ($p<0.001$) with a standardised difference in means of -0.543 (95% CI [0.232, 0.854]). From the summary statistics (Table 5), the I^2 value of 50.54 suggests moderate heterogeneity between outcomes of the studies included in the meta-analysis but due to a p-value of 0.088 heterogeneity between outcomes is statistically non-significant. The direction of effect was generally the same across all studies, however, the 2 exercise groups within the study by Kerling et al. (2015) produced non-significant increases in $Lactate_{MAX}$ following exercise intervention.

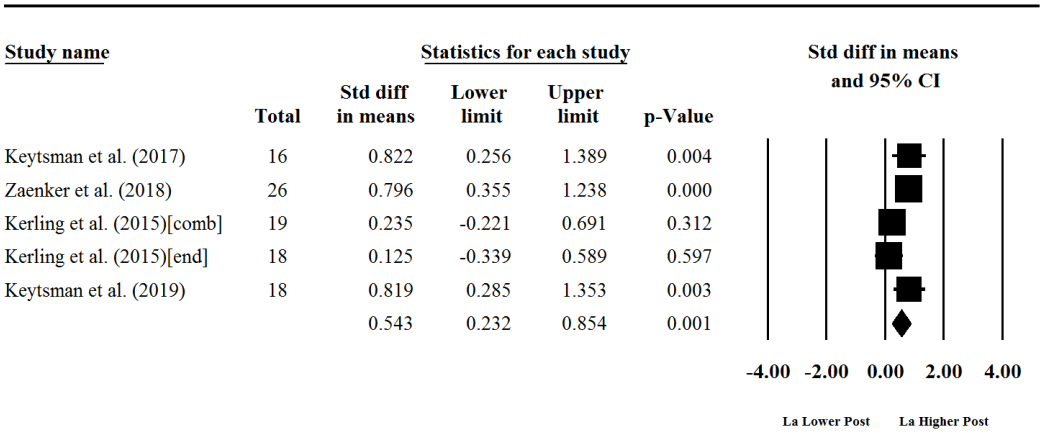


Fig. 6 Forest plot of maximal lactate concentrations obtained in PwMS before and after completion of exercise intervention, with the final row representing results of data analysis synthesis. Abbreviations: Lactate (La), combined training intervention group ([comb]), endurance training intervention group ([end]).

4.0 Discussion

4.1 Main findings

The objectives of this systematic review and meta-analysis were to compare blood lactate concentrations at rest and during exercise in PwMS to HC, and to assess changes in exertional blood lactate production following an exercise intervention. The main findings are (1) resting blood lactate is significantly higher in PwMS than in HC, (2) maximal lactate is lower in PwMS than in HC, and (3) in PwMS exercise training results in reductions in submaximal and increases in maximal lactate concentrations. Meta-regression analysis to test for associations was precluded, however, as there were insufficient studies for them to be performed. This is the first systematic review and meta-analysis on these topics, providing novel findings across all variables and indicating multiple potential pathways for future research in MS symptom development, disease severity and intervention impact.

4.2 Lactate in PwMS compared to HC

4.2.1 $Lactate_{REST}$

PwMS display significantly higher $Lactate_{REST}$ compared to HC with a large effect size (Figure 2). This supports the conclusions drawn by Amorini et al. [7], who reported higher resting lactate concentrations in all MS types included within their study compared to HC (RR, PP, SP; see Section 3.3). These findings provide support for blood lactate to potentially find use as a biomarker for MS and the hypothesis of an association between mitochondrial dysfunction and MS. It is also important to note that while the effect size was reduced on the removal of one study [7] due to the relative statistical power of this study's large sample size, the elevation of $Lactate_{REST}$ in PwMS remained both large and statistically significant, further strengthening the integrity of these results.

538

539 The mechanism behind this is unclear and may arise from a variety of causes. From the limited data
540 provided for $VO2_{REST}$ in PwMS compared to HC [17,28,36], there seems to be little evidence to suggest
541 that the differences in resting lactate are caused by differences in oxygen consumption in PwMS
542 compared to HC, though evidence is limited and such confounding factors are not completely
543 eliminated. It is also unlikely that $Lactate_{REST}$ is affected by differences in fitness levels between PwMS
544 and HC given previous research has reported no significant differences in $Lactate_{REST}$ between trained
545 and untrained healthy cohorts [39–41]. Elevated lactate at rest has been recorded as a result of
546 mitochondrial dysfunction [42] and atherosclerosis [43], as well as others including ischaemia,
547 malignancy and trauma [44]. It has been hypothesized in previous literature that mitochondrial
548 dysfunction may be a contributor to progression of MS [45–47], as such, the results of the meta-
549 analysis (Figure 2) may strengthen the potential link between MS and mitochondrial dysfunction.
550 Conversely, however, the elevation of resting blood lactate may find a root in impaired peripheral
551 vascular function. It has been reported that elevated pulse pressure, a marker of arterial stiffness, is
552 significantly associated with a decreased distance during a 6-minute walk test (6-MWT) in PwMS [48],
553 a measure which correlates strongly with EDSS score [49]. It has also been reported that vascular
554 comorbidities are associated with impaired mobility in PwMS [50]. Given the reported association with
555 lactate and atherosclerosis in previous literature [43] and the association between Multiple Sclerosis
556 and elevated risk of cardiovascular disease [51,52], elevated lactate in PwMS may be an indicator of
557 secondary vascular effects, as well as or instead of primary neurological effects of MS. At this stage of
558 research, however, it is not possible to make firm conclusions.

559

560 More studies are required to measure the association between EDSS score and blood lactate at rest.
561 Amorini et al. [7] reported a moderate correlation between resting blood lactate and EDSS score (R^2 =
562 0.419; $p < 0.0001$) and strong correlation between resting blood lactate and median EDSS score in
563 PwMS ($R^2 = 0.972$; $p < 0.0001$) but none of the other studies found in this systematic review compared

564 resting blood lactate between groups with higher and lower EDSS scores. A useful direction of future
565 research would be to focus on intra-individual changes in resting blood lactate concentration for
566 PwMS throughout disease progression, undergoing relapse and/or fatigue and the specificity and
567 sensitivity of blood lactate as a biomarker of MS.
568
569 4.2.2 $Lactate_{MAX}$

570 PwMS have significantly lower lactate production at maximal intensity exercise levels compared to HC
571 (Figure 3). Unfortunately, there were insufficient statistical analyses of power output during the CPETs
572 used to measure $Lactate_{MAX}$ and the authors of this paper were subsequently unable to establish the
573 contribution of differences in power output to the differences in $Lactate_{MAX}$ between PwMS and HC.
574 One study did report non-significant differences between power output at maximum intensity
575 exercise in PwMS compared to HC [33], but the small sample sizes within the study (8 PwMS and 7
576 HC) mean the results cannot be generalised to the wider population with confidence. Previous
577 literature with larger sample sizes have confirmed significantly reduced peak power output in PwMS
578 compared to HC [53,54]. Additionally, the evidence from papers reporting $VO2_{MAX}$ in PwMS and HC
579 [31–33,38], which reported higher $VO2_{MAX}$ values in HC compared to PwMS suggests a reduced
580 metabolic demand from musculoskeletal tissues in MS compared to HC, a finding supported by a
581 previous systematic review and meta-analysis on aerobic capacity on PwMS [55]. As such, it may not
582 be unreasonable to conclude that reduced metabolic demand on the muscles due to reduced muscle
583 activation is a potentially significant mechanism behind the difference in power output at maximum
584 intensity exercise and may have an impact on $Lactate_{MAX}$ response. This would be supported by
585 previous literature reporting reduced motor unit activation and recruitment as a symptom of MS [56].

586 Additionally, a reduced $Lactate_{MAX}$ response in higher EDSS scores compared to lower EDSS scores was
587 observed (Figure 4). As above, the mechanism behind this phenomenon is likely to be affected by
588 increasing impairment of motor unit recruitment with increasing severity of MS symptoms [56]. This

589 is supported by the data from the literature in this review (Section 3.10.2) as well as in other literature
590 [53] reporting significantly lower peak power output in higher EDSS scoring groups of PwMS compared
591 to their lower EDSS scoring counterparts. The presence of this reduced $Lactate_{MAX}$ response in PwMS
592 and its further reduction with increasing EDSS score also suggests potential for $Lactate_{MAX}$ to function
593 as a biomarker itself. What remains unclear is if other hypothesized mechanisms of MS progression,
594 e.g. mitochondrial dysfunction, are also playing a contributing factor to the impairment of $Lactate_{MAX}$
595 response in PwMS both compared to HC and with increasing disease severity. Future research is
596 required to clarify the magnitude of this impairment of $Lactate_{MAX}$ due to MS symptoms as well as the
597 precise mechanisms by which this impairment occurs.

598 4.2.3 Lactate response range

599 Taken together, the findings of the meta-analyses of $Lactate_{REST}$ and $Lactate_{MAX}$ (Figures 2 and 3)
600 illustrate an overall lactate range compression from resting state to maximal exertion in PwMS
601 compared to HC. Moreover, the findings that $Lactate_{MAX}$ is further reduced in PwMS with higher EDSS
602 when compared to those with lower EDSS scoring PwMS (Figure 4) show that $Lactate_{REST}$ – $Lactate_{MAX}$
603 range compression is further exacerbated by MS disease progression. The occurrence of this
604 compression is largely supportive of the suggestion by Amorini et al. [7] that resting blood lactate in
605 PwMS is correlated EDSS score, but future research into lactate mechanics in PwMS is still warranted
606 to confirm the extent of lactate range compression in PwMS and if it could have any clinical relevance
607 in measuring severity of disease progression.
608
609 4.3 Effects of exercise intervention on Lactate in PwMS
610
611 4.3.1 $Lactate_{REST}$
612

Only 2 studies [19,25] reported the effects of training $Lactate_{REST}$ in PwMS and so there were not enough studies to perform a meta-analysis on this variable. However, both studies reported significant within-group falls in resting blood lactate following a 6-month training intervention, suggesting that blood lactate in PwMS may be reduced in response to training. Given the association between EDSS and $Lactate_{REST}$ reported by Amorini et al. [7], a key question for future research is whether changes in $Lactate_{REST}$ translate to alterations in EDSS or symptom severity. Without assessment of resting lactate, it is impossible to determine if any changes in symptom severity occur in tandem with alterations in metabolic processes.

4.3.2 $Lactate_{SUB\text{-}MAX}$

A meta-analysis of lactate response to submaximal intensity exercise (Figure 5) showed a reduction in lactate response in PwMS following and exercise intervention. It is worth noting that for at least 2 of the 3 studies investigating this lactate response [25,26], power output during the sub-maximal intensity exercise test was kept the same during baseline and post-intervention. Should this be the case, it would demonstrate that the causes the change in $Lactate_{SUB\text{-}MAX}$ response following an exercise intervention were not down to changes in motor unit and muscular activation but instead down to other potential factors associated with exercise training, such as increasing mitochondrial density, size and metabolic enzyme activity, increased capillarisation and flow-mediated dilation, as well as others [57–59]. However, given a lack of change in $VO2_{SUB\text{-}MAX}$ for the cohorts within the studies which reported it [25,26] it is difficult to confirm if this is the case or if such changes to $Lactate_{SUB\text{-}MAX}$ in response to an exercise intervention were clinically meaningful within this cohort. Future research would be well placed to investigate the precise mechanisms of physiological adaptations to exercise in PwMS as well as the degree to which sub-maximal lactate response adaptations are associated with changes in functional capacity and quality of life.

639 4.3.3 $Lactate_{MAX}$

640

641 PwMS demonstrate a significant increase in $Lactate_{MAX}$ response following a structured exercise
642 programme over the course of at least 3 months (Figure 6), demonstrating that aerobic capacity in
643 PwMS can be improved through a structured training programme [55]. Given the variable nature of
644 each study's training programme, with 5/6 studies incorporating some combination of endurance,
645 resistance and HIIT training, it is difficult to comment on the precise mechanisms by which adaptations
646 were made to increase $Lactate_{MAX}$ and where training modalities overlap. Nevertheless, it seems likely
647 that exercise intensity is an important variable in predicting the response. For example, of studies
648 using HITT [23,24,27], the greatest changes in $Lactate_{MAX}$ (Figure 6) were observed, suggesting a
649 potential pattern of HIIT adoption and expansion of the compressed Lactate range observed in PwMS.
650 It has been shown in the general population that HIIT induces greater increases in mitochondrial
651 content, enzyme activity, potentially skeletal muscle recruitment compared to moderate intensity
652 training [60,61], and that increased lactate buffering capacity in muscle is observed in both sprint
653 interval training and continuous training [62]. Additionally, considering 3 studies out of 4 [23,24,27]
654 reported significant increases in peak power output in PwMS and 2 studies out of 3 [24,31,38]
655 reported significant increases in $VO2_{MAX}$ in PwMS following an exercise intervention, it can be
656 reasonably concluded that increased skeletal muscle recruitment and enhanced metabolic activity had
657 some effect on the increase in $Lactate_{MAX}$ response. Further research is needed to determine if HIIT is
658 indeed more effective at increasing $Lactate_{MAX}$ concentration in PwMS and whether the causes by
659 which this occurs is due to any of the other aforementioned mechanisms. It also remains unclear how
660 the adaptability of $Lactate_{MAX}$ response is affected in PwMS compared to HC, as no studies were found
661 to investigate these variables. As such, further research into these gaps in the literature are warranted
662 to expand this knowledgebase.

663

It may also be noted that, while the data is too limited to draw confident conclusions, increases in $Lactate_{MAX}$ appear to be associated with increases in both power output and $VO2_{MAX}$ in PwMS. As such, as has been the case for the researchers of this paper investigating health and fitness-related variables in PwMS during the COVID-19 pandemic, Lactate testing may be an alternative method of remotely measuring changes in physical fitness in PwMS in response to an exercise intervention during periods of limited availability of physical contact with researchers or clinicians. This is due to its widespread availability to researchers, relatively low equipment costs and potential suitability for PwMS to be tested at home by either themselves or a carer.

4.4 Limitations of the literature

Studies generally had a low sample size, with a third of studies involving less than 30 participants [23–25,28,33,36], which inherently reduces generalizability of results to the wider population of interest and risks detecting large sample sizes which cannot be replicated in future studies. A greater number of studies would allow for a more nuanced analysis, including meta-regression or moderator analysis to identify areas underpinning any heterogeneity and to minimise influence of results that may have been duplicated to some degree by repeated use of cohort data across different studies. More studies would also allow for meta-analyses on the effect of exercise on $Lactate_{REST}$ in PwMS or the differences in $Lactate_{SUB\text{-}MAX}$ between PwMS and HC exercising at the same power output; both of which were impossible due to the limited number of studies investigating them.

It would be beneficial if more studies provided more complete data with regard to power output and VO2 during their respective testing conditions. There was limited scope for this review to investigate potential correlations or confounding factors regarding the interactions between Lactate concentration, power output and VO2; both of which could affect the suitability of blood Lactate concentration as either a biomarker or as a suitable alternative to ore complex physiological

690 measurements of health and fitness in PwMS during times of limited clinician or researcher contact
691 with PwMS.
692
693 Differences in testing and training protocols, as seen in Tables 2, 3 and 4, made comparisons between
694 studies difficult. The result of these issues was moderately or highly heterogeneity between studies,
695 especially for studies comparing $Lactate_{REST}$ and $Lactate_{MAX}$ between PwMS and HC. An example of this
696 was in testing site utilised for lactate measurement and the equipment used for lactate testing, where
697 5 studies failed to adequately describe one or both of these aspects of methodology [23,24,30,31,33].
698 The most commonly utilised site, the fingertip, was only used by half of the studies included in this
699 review [17,19,25,26,28,29,32,33,36]. It has been shown that the testing site for blood lactate
700 measurement makes a significant difference to the concentration measured [63–65] and so the
701 variability of testing site protocol in studies included in this review may have impacted the reliability
702 of comparisons between studies. Additionally, 7 studies reported utilising laboratory-based lactate
703 measurement protocols [7,18,30–32,34,35] and 9 reported using Accutrend Plus and Lactate Pro
704 portable devices [17,19,25–29,33,36]. While portable lactate testing consumer devices, eg. Accutrend
705 and Lactate Pro, have been shown to have reasonable accuracy compared with a laboratory-based
706 method [66], variability in testing equipment may have impacted results across studies. Finally,
707 findings from Keytsman et al. [23,27] showed a significantly greater blood lactate concentration in
708 PwMS during post-CPET recovery ($Lactate_{PEAK}$) compared to the moment of CPET cessation
709 ($Lactate_{MAX}$). Given there were 5 different methods overall for timing $Lactate_{MAX}$ measurements
710 (Section 3.9.3), the differences in post-CPET lactate test timing across studies was a potential cause of
711 variability in results and would benefit from more uniform measurement procedure in future.
712
713 In terms of reporting EDSS, only one paper [24] provided EDSS data in a form as to be able to establish
714 interquartile (IQ) range of the EDSS scores of participants, with the rest reporting in the form of mean
715 ± SD, or mean ± SE. EDSS is not a normally distributed score, therefore such reporting measures

provide an incomplete report of EDSS score of the participant group with MS for further research and it is the recommendation of the authors of this paper to report IQ range of EDSS to provide a clearer picture of cohorts used within the study.

4.5 Suggestions for future research

Given the above, and given the findings in this paper and by Amorini et al. [7], more studies are required that have blood lactate concentration, changes in blood lactate concentration (if undergoing a training intervention) and EDSS score/symptom severity as primary outcomes rather than secondary data reported only as part of a standard exercise /CPET test. With this, longer training interventions, greater participant numbers and uniform testing procedures, a more nuanced understanding of blood lactate in PwMS and the mechanisms behind its metabolic response and biomarker reliability would be available for research.

4.6 Limitation of this review

This review was limited primarily by the low number of studies performing blood lactate testing in PwMS during each condition of rest, and submaximal and maximal exercise intensities. As such, meta-analyses either could not be performed for a particular condition (eg. $Lactate_{REST}$ pre- vs. post-intervention) or were performed using 3-5 studies. Limiting the publication language to English and exclusion of non-peer-reviewed literature (e.g. grey literature) means that some studies may have been missed, however the authors employed a rigorous search strategy to ensure as many studies as possible were included in the overall review.

5.0 Conclusion

Blood lactate occurs in higher concentrations during rest in PwMS compared to HC and $Lactate_{MAX}$ is lower in PwMS compared to HC, with higher EDSS scoring groups displaying lower $Lactate_{MAX}$ concentrations than lower EDSS scoring groups. With a long-term training intervention, $Lactate_{REST}$ and $Lactate_{SUB\text{-}MAX}$ concentrations may decrease, while $Lactate_{MAX}$ concentrations may increase, compared to baseline values. There is evidence to suggest these changes arise from both increased skeletal muscle recruitment as well as adaptations to factors such as capillary structure and mitochondrial number and efficiency. Given the purported role of lactate as both a biomarker of disease severity, and a prognostic indicator of future relapse, more work is needed to elucidate the association between resting and exertional metabolism and ongoing disease processes. The primary limitations to the literature were small sample sizes and heterogeneity of lactate testing protocols due to lactate testing often being a secondary outcome measure, while the primary limitation to this systematic review and meta-analysis was a low number of studies included. Future research would be worthwhile to investigate the changes in blood lactate during fatigue and relapse.

6.0 References

1. Dobson R, Giovannoni G. Multiple sclerosis – a review. Eur J Neurol. 2019;26:27–40.

2. Lublin FD, Reingold SC, Cohen JA, Cutter GR, Sørensen PS, Thompson AJ, et al. Defining the clinical course of multiple sclerosis: the 2013 revisions. Neurology. 2014;83:278–86.

3. Kurtzke JF. Rating neurologic impairment in multiple sclerosis: An expanded disability status scale (EDSS). Neurology. 1983;33:1444–1444.

4. Pucino V, Bombardieri M, Pitzalis C, Mauro C. Lactate at the crossroads of metabolism, inflammation, and autoimmunity. Eur J Immunol. 2017;47:14–21.

5. Paling D, Golay X, Wheeler-Kingshott C, Kapoor R, Miller D. Energy failure in multiple sclerosis and its investigation using MR techniques. J Neurol. 2011;258:2113–27.

6. Albanese M, Zagaglia S, Landi D, Boffa L, Nicoletti CG, Marciani MG, et al. Cerebrospinal fluid lactate is associated with multiple sclerosis disease progression. J Neuroinflammation [Internet]. 2016 [cited 2020 Jun 26];13. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4750170/

7. Amorini AM, Nociti V, Petzold A, Gasperini C, Quartuccio E, Lazzarino G, et al. Serum lactate as a novel potential biomarker in multiple sclerosis. Biochim Biophys Acta-Mol Basis Dis. 2014;1842:1137–43.

8. Housley WJ, Pitt D, Hafler DA. Biomarkers in multiple sclerosis. Clin Immunol. 2015;161:51–8.

9. Moran P, Prichard JG, Ansley L, Howatson G. The Influence of Blood Lactate Sample Site on Exercise Prescription. J Strength Cond Res. 2012;26:563–7.

10. Aduen J. The Use and Clinical Importance of a Substrate-Specific Electrode for Rapid Determination of Blood Lactate Concentrations. JAMA J Am Med Assoc. 1994;272:1678.

11. Baker JS, McCormick MC, Robergs RA. Interaction among Skeletal Muscle Metabolic Energy Systems during Intense Exercise. J Nutr Metab. 2010;2010:1–13.

12. Svedahl K, MacIntosh BR. Anaerobic Threshold: The Concept and Methods of Measurement. Can J Appl Physiol. NRC Research Press; 2003;28:299–323.

13. Figueira TR, Caputo F, Pelarigo JG, Denadai BS. Influence of exercise mode and maximal lactate-steady-state concentration on the validity of OBLA to predict maximal lactate-steady-state in active individuals. J Sci Med Sport. 2008;11:280–6.

14. Cerezuela-Espejo V, Courel-Ibáñez J, Morán-Navarro R, Martínez-Cava A, Pallarés JG. The Relationship Between Lactate and Ventilatory Thresholds in Runners: Validity and Reliability of Exercise Test Performance Parameters. Front Physiol. 2018;9:1–10. Available from: https://www.frontiersin.org/articles/10.3389/fphys.2018.01320/full

15. Philp A. Lactate - a signal coordinating cell and systemic function. J Exp Biol. 2005;208:4561–75.

790 16. San-Millán I, Brooks GA. Assessment of Metabolic Flexibility by Means of Measuring Blood
791 Lactate, Fat, and Carbohydrate Oxidation Responses to Exercise in Professional Endurance Athletes
792 and Less-Fit Individuals. Sports Med. 2018;48:467–79.

793 17. Hansen D, Wens I, Kosten L, Verboven K, Eijnde BO. Slowed Exercise-Onset Vo(2) Kinetics During
794 Submaximal Endurance Exercise in Subjects With Multiple Sclerosis. Neurorehabil Neural Repair.
795 2013;27:87–95.

796 18. Kerling A, Keweloh K, Tegtbur U, Kueck M, Grams L, Horstmann H, et al. Effects of a Short
797 Physical Exercise Intervention on Patients with Multiple Sclerosis (MS). Int J Mol Sci. 2015;16:15761–
798 75.

799 19. Wens I, Hansen D, Verboven K, Deckx N, Kosten L, Stevens ALM, et al. Impact of 24 Weeks of
800 Resistance and Endurance Exercise on Glucose Tolerance in Persons with Multiple Sclerosis: Am J
801 Phys Med Rehabil. 2015;94:838–47.

802 20. WHO ACT Accelerator Ethics & Governance Working Group, Health Ethics & Governance,
803 Working Group on Ethics and COVID-19. Ethical standards for research during public health
804 emergencies: Distilling existing guidance to support COVID-19 R&D [Internet]. World Health
805 Organization; 2020 [cited 2021 Apr 2]. Available from:
806 https://apps.who.int/iris/bitstream/handle/10665/331507/WHO-RFH-20.1-eng.pdf

807 21. Moher D, Liberati A, Tetzlaff J, Altman DG, PRISMA Group. Preferred reporting items for
808 systematic reviews and meta-analyses: the PRISMA statement. BMJ. 2009;339:b2535.

809 22. Downes MJ, Brennan ML, Williams HC, Dean RS. Development of a critical appraisal tool to assess
810 the quality of cross-sectional studies (AXIS). BMJ Open. 2016;6:e011458.

811 23. Keytsman C, Hansen D, Wens I, O. Eijnde B. Impact of high-intensity concurrent training on
812 cardiovascular risk factors in persons with multiple sclerosis – pilot study. Disabil Rehabil.
813 2017;41:430–5.

814 24. Zaenker P, Favret F, Lonsdorfer E, Muff G, de Seze J, Isner-Horobeti M-E. High-intensity interval
815 training combined with resistance training improves physiological capacities, strength and quality of
816 life in multiple sclerosis patients: a pilot study. Eur J Phys Rehabil Med. 2018;54:58–67.

817 25. Hansen D, Wens I, Keytsman C, Eijnde BO, Dendale P. Is long-term exercise intervention effective
818 to improve cardiac autonomic control during exercise in subjects with multiple sclerosis? A
819 randomized controlled trial. Eur J Phys Rehabil Med. 2015;51:223–31.

820 26. Hansen D, Wens I, Keytsman C, Verboven K, Dendale P, Eijnde BO. Ventilatory function during
821 exercise in multiple sclerosis and impact of training intervention: cross-sectional and randomized
822 controlled trial. Eur J Phys Rehabil Med. 2015;51:557–68.

823 27. Keytsman C, Van Noten P, Spaas J, Nieste I, Van Asch P, Eijnde BO. Periodized home-based
824 training: A new strategy to improve high intensity exercise therapy adherence in mildly affected
825 patients with Multiple Sclerosis. Mult Scler Relat Disord. 2019;28:91–7.

826 28. Cleland BT, Ingraham BA, Pitluck MC, Woo D, Ng AV. Reliability and Validity of Ratings of
827 Perceived Exertion in Persons With Multiple Sclerosis. Arch Phys Med Rehabil. 2016;97:974–82.

828 29. Hansen D, Wens I, Dendale P, Eijnde BO. Exercise-onset heart rate increase is slowed in multiple
829 sclerosis patients: Does a disturbed cardiac autonomic control affect exercise tolerance?
830 Neurorehabilitation. 2013;33:139–46.

831 30. Juybari KB, Ebrahimi G, Momeni Moghaddam MA, Asadikaram G, Torkzadeh-Mahani M, Akbari
832 M, et al. Evaluation of serum arsenic and its effects on antioxidant alterations in relapsing-remitting
833 multiple sclerosis patients. Mult Scler Relat Disord. 2018;19:79–84.

834 31. Kerling A, Keweloh K, Tegtbur U, Kück M, Grams L, Horstmann H, et al. Physical capacity and
835 quality of life in patients with multiple sclerosis. NeuroRehabilitation. 2014;35:97–104.

836 32. Langeskov-Christensen M, Langeskov-Christensen D, Overgaard K, Møller AB, Dalgas U. Validity
837 and reliability of VO2-max measurements in persons with multiple sclerosis. J Neurol Sci.
838 2014;342:79–87.

839 33. Larson RD, McCully KK, Larson DJ, Pryor WM, White LJ. Lower-limb performance disparities:
840 Implications for exercise prescription in multiple sclerosis. J Rehabil Res Dev. Baltimore, Maryland:
841 VA Prosthetics Research & Development Center; 2014;51:1537–43.

842 34. Penesova A, Vlcek M, Imrich R, Vernerova L, Marko A, Meskova M, et al. Hyperinsulinemia in
843 newly diagnosed patients with multiple sclerosis. Metab Brain Dis. 2015;30:895–901.

844 35. Schlüter K, Maier J, Patra S, Gold SM, Heesen C, Schulz K-H. Aberrant peak lactate response in
845 MS. NeuroRehabilitation. 2017;41:811–22.

846 36. ’t Eijnde BO, Keytsman C, Wens I, Hansen D. Whole-body cooling does not compromise muscle
847 oxidative capacity in subjects with multiple sclerosis. Neurorehabilitation. 2014;35:805–11.

848 37. Larson RD, McCully KK, Larson DJ, Pryor WM, J.White L. Bilateral differences in lower-limb
849 performance in individuals with multiple sclerosis. J Rehabil Res Dev. 2013;50:215.

850 38. Keytsman C, Van Noten P, Spaas J, Nieste I, Van Asch P, Eijnde BO. Periodized home-based
851 training: A new strategy to improve high intensity exercise therapy adherence in mildly affected
852 patients with Multiple Sclerosis. Mult Scler Relat Disord. 2019;28:91–7.

853 39. Collomp K, Ahmaidi S, Chatard JC, Audran M, Préfaut Ch. Benefits of caffeine ingestion on sprint
854 performance in trained and untrained swimmers. Eur J Appl Physiol. 1992;64:377–80.

855 40. Stone MH, Pierce K, Godsen R, Wilson GD, Blessing D, Rozenek R, et al. Heart Rate and Lactate
856 Levels During Weight-Training Exercise in Trained and Untrained Men. Phys Sportsmed. 1987;15:97–
857 105.

858 41. Lehmann M, Keul J. Free Plasma Catecholamines, Heart Rates, Lactate Levels, and Oxygen
859 Uptake in Competition Weight Lifters, Cyclists, and Untrained Control Subjects* **. Int J Sports Med.
860 1986;07:18–21.

861 42. Debray F-G, Mitchell GA, Allard P, Robinson BH, Hanley JA, Lambert M. Diagnostic Accuracy of
862 Blood Lactate-to-Pyruvate Molar Ratio in the Differential Diagnosis of Congenital Lactic Acidosis. Clin
863 Chem. 2007;53:916–21.

864 43. Shantha GPS, Wasserman B, Astor BC, Coresh J, Brancati F, Sharrett AR, et al. Association of
865 blood lactate with carotid atherosclerosis: the Atherosclerosis Risk in Communities (ARIC) Carotid
866 MRI Study. Atherosclerosis. 2013;228:249–55.

867 44. Wardi G, Brice J, Correia M, Liu D, Self M, Tainter C. Demystifying Lactate in the Emergency
868 Department. Ann Emerg Med. 2020;75:287–98.

869 45. Barcelos IP de, Troxell RM, Graves JS. Mitochondrial Dysfunction and Multiple Sclerosis. Biology.
870 2019;8.

871 46. Campbell GR, Mahad DJ. Mitochondria as Crucial Players in Demyelinated Axons: Lessons from
872 Neuropathology and Experimental Demyelination. Autoimmune Dis. 2011;2011:1–9.

873 47. Su K, Bourdette D, Forte M. Mitochondrial dysfunction and neurodegeneration in multiple
874 sclerosis. Front Physiol [Internet]. 2013 [cited 2020 Jul 3];4. Available from:
875 http://journal.frontiersin.org/article/10.3389/fphys.2013.00169/abstract

876 48. Heffernan KS, Ranadive S, Weikert M, Lane A, Yan H, Fernhall B, et al. Pulse pressure is
877 associated with walking impairment in multiple sclerosis. J Neurol Sci. 2011;309:105–9.

878 49. Goldman MD, Marrie RA, Cohen JA. Evaluation of the six-minute walk in multiple sclerosis
879 subjects and healthy controls. Mult Scler J. 2008;14:383–90.

880 50. Marrie RA, Rudick R, Horwitz R, Cutter G, Tyry T, Campagnolo D, et al. Vascular comorbidity is
881 associated with more rapid disability progression in multiple sclerosis. Neurology. 2010;74:1041–7.

882 51. Christiansen CF, Christensen S, Farkas DK, Miret M, Sørensen HT, Pedersen L. Risk of arterial
883 cardiovascular diseases in patients with multiple sclerosis: a population-based cohort study.
884 Neuroepidemiology. 2010;35:267–74.

885 52. Jadidi E, Mohammadi M, Moradi T. High risk of cardiovascular diseases after diagnosis of
886 multiple sclerosis. Mult Scler Houndmills Basingstoke Engl. 2013;19:1336–40.

887 53. Klaren RE, Sandroff BM, Fernhall B, Motl RW. Comprehensive Profile of Cardiopulmonary
888 Exercise Testing in Ambulatory Persons with Multiple Sclerosis. Sports Med. 2016;46:1365–79.

889 54. Motl RW, Fernhall B. Accurate Prediction of Cardiorespiratory Fitness Using Cycle Ergometry in
890 Minimally Disabled Persons With Relapsing-Remitting Multiple Sclerosis. Arch Phys Med Rehabil.
891 2012;93:490–5.

892 55. Langeskov-Christensen M, Heine M, Kwakkel G, Dalgas U. Aerobic Capacity in Persons with
893 Multiple Sclerosis: A Systematic Review and Meta-Analysis. Sports Med. 2015;45:905–23.

894 56. White LJ, Dressendorfer RH. Exercise and Multiple Sclerosis. Sports Med. 2004;24.

895 57. Drake JC, Wilson RJ, Yan Z. Molecular mechanisms for mitochondrial adaptation to exercise
896 training in skeletal muscle. FASEB J. 2016;30:13–22.

897 58. Green DJ, Hopman MTE, Padilla J, Laughlin MH, Thijssen DHJ. Vascular Adaptation to Exercise in
898 Humans: Role of Hemodynamic Stimuli. Physiol Rev. 2017;97:495–528.

899 59. Lundby C, Jacobs RA. Adaptations of skeletal muscle mitochondria to exercise training. Exp
900 Physiol. 2016;101:17–22.

901 60. MacInnis MJ, Gibala MJ. Physiological adaptations to interval training and the role of exercise
902 intensity. J Physiol. 2017;595:2915–30.

903 61. Vøllestad NK, Blom PCS. Effect of varying exercise intensity on glycogen depletion in human
904 muscle fibres. Acta Physiol Scand. 1985;125:395–405.

905 62. Gibala MJ, Little JP, Van Essen M, Wilkin GP, Burgomaster KA, Safdar A, et al. Short-term sprint
906 interval *versus* traditional endurance training: similar initial adaptations in human skeletal muscle
907 and exercise performance: Rapid adaptations to sprint or endurance training in humans. J Physiol.
908 2006;575:901–11.

909 63. El-Sayed MS, George KP, Wilkinson D, Mullan N, Fenoglio R, Flannigan J. Fingertip and venous
910 blood lactate concentration in response to graded treadmill exercise. J Sports Sci. 1993;11:139–43.

911 64. Felippe LC, Ferreira GA, De-Oliveira F, Pires FO, Lima-Silva AE. Arterialized and venous blood
912 lactate concentration difference during different exercise intensities. J Exerc Sci Fit. 2017;15:22–6.

913 65. Feliu J, Ventura JL, Segura R, Rodas G, Riera J, Estruch A, et al. Differences between lactate
914 concentration of samples from ear lobe and the finger tip. J Physiol Biochem. 1999;55:333–9.

915 66. Baldari C, Bonavolontà V, Emerenziani GP, Gallotta MC, Silva AJ, Guidetti L. Accuracy, reliability,
916 linearity of Accutrend and Lactate Pro versus EBIO plus analyzer. Eur J Appl Physiol. 2009;107:105–
917 11.

918

919