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*Original Article*

# The relationship between skeletal muscle mitochondrial citrate synthase activity and whole body oxygen uptake adaptations in response to exercise training

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**Abstract:** Citrate synthase (CS) activity is a validated biomarker for mitochondrial density in skeletal muscle. CS activity is also used as a biochemical marker of the skeletal muscle oxidative adaptation to a training intervention, and a relationship between changes in whole body aerobic capacity and changes in CS activity is often assumed. However, this relationship and absolute values of CS and maximal oxygen uptake ($\dot{V}O_{2max}$) has never been assessed across different studies. A systematic PubMed search on literature published from 1983 to 2013 was performed. The search profile included: *citrate, synthase, human, skeletal, muscle, training, not electrical stimulation, not in-vitro, not rats*. Studies that reported changes in CS activity and $\dot{V}O_{2max}$ were included. Different training types and subject populations were analyzed independently to assess correlation between relative changes in $\dot{V}O_{2max}$ and CS activity. 70 publications with 97 intervention groups were included. There was a positive ($r$ = 0.45) correlation ($P$ < 0.001) between the relative change in $\dot{V}O_{2max}$ and the relative change in CS activity. All reported absolute values of CS and $\dot{V}O_{2max}$ did not correlate ($r$ = -0.07, $n$ = 148, $P$ = 0.4). Training induced changes in whole body oxidative capacity is matched by changes in muscle CS activity in a nearly 1:1 relationship. Absolute values of CS across different studies cannot be compared unless a standardized analytical method is used by all laboratories.

**Keywords:** Citrate synthase, endurance training, high-intensity interval training, human skeletal muscle, maximal oxygen uptake

## Introduction

Cardiac output and not skeletal muscle enzymatic activity is the limiting factor to aerobic performance in healthy people [1]. Nevertheless, adequate muscle enzymatic activity in e.g. glycolysis and Krebs cycle is necessary for a high performance and maximal oxygen uptake ($\dot{V}O_{2max}$). Enzymatic activity in human skeletal muscle, and in particular citrate synthase (CS) activity, has been used a marker of cellular oxidative capacity and mitochondrial density following a training regimen [2, 3]. These enzyme activities are highly adaptable to aerobic training and during exercise a high enzymatic capacity is essential for optimal performance during aerobic exercise [4]. While these characteristics of oxidative enzymes have been known for decades, there is a lack of literature on the relationship between training induced changes in CS activity and whole body $\dot{V}O_{2max}$. The relationship between $\dot{V}O_{2max}$ and CS activity may provide information on whether cardiovascular and local metabolic adaptations are coupled (i.e. do both systems adapt together), and in which subjects or training types does one change more than the other if one is more important to changes in $\dot{V}O_{2max}$ than the other?

A relationship between changes in $\dot{V}O_{2max}$ and changes in CS activity is assumed and often based on observations from classical endurance training (ET) studies with low intensity and long duration. Most of these studies have shown increased CS activity after training [i.e. 5, 6, see **Table 1**], with seemingly similar effect in both genders (Coggan et al., 1992). In the last decade high-intensity interval training (HIIT) has received wide interest as a time-efficient training modality, using a very high intensity for

a very short duration. HIIT has been shown to increase CS activity in most but not all studies [7-11].

Lower CS activity has been reported in elderly compared to equally active young subjects [12]. This has also been observed in a cross-sectional study where CS activity was lower in both sedentary and active elderly subjects compared to young sedentary and active subjects matched for daily activity by the Baecke questionnaire [13] but with a lower $\dot{V}O_{2max}$ per kg fat free mass (FFM) in the elderly subjects [14].

CS activity have been shown to be lower in a group of obese, insulin resistant subjects compared to a group of obese insulin sensitive subjects matched for $\dot{V}O_{2max}$ per kg FFM, but in none of the groups an increase in CS activity was seen in response to 6 weeks aerobic endurance training despite increases in $\dot{V}O_{2max}$ per kg FFM [15]. Thus, the metabolic state of subject may challenge the relationship between training induced changes in CS activity and in $\dot{V}O_{2max}$.

Analysis of CS in skeletal muscle requires relative small biopsy samples (approximately 15 mg w.w.) and the assay has a relatively low inter- and intra assay variation (below 5% in our laboratory), and the analysis can be done on frozen samples. However, methodological variations and differences in preparation of the biopsies between the different studies is a possible concern. CS activity is traditionally analyzed by the methods described by Lowry and Passonneau [16] or by Srere [17]. The latter is based on a reaction between the thiolgroup in acetyl-CoA which react with Ellman's reagent (5, 5'-dithiobis-(2-nitrobenzoic acid (DTNB)), which is measured spectrophotometrically [17]. The method by Lowry and Passoneau is based on the conversion of malate to oxaloacetate by reduction of $NAD^+$ to NADH, where the formation of NADH is linear to the CS activity [16]. In this method NADH may be measured both spectrophotometrically and fluorometrically. Different laboratories use these methods with various modifications, different reagents or temperatures (range: 25-37°C) resulting in possible differences in CS activity between laboratories. Furthermore, the analysis may either be done on untreated tissue (wet weight) or tissue that has been freeze-dried and dissected free of visible connective tissue, blood and adipose tissue (dry weight). Using dry weight ensures that the analysis is done primarily on muscle tissue, and not on adipose or connective tissue, which improves the validity of the result. In addition to the analytical considerations, the time from last exercise bout to the biopsy sampling is of importance. Tonkonogi and colleagues showed that CS activity is increased immediately after acute exercise (30 sec. after cessation of exercise) [18]. This finding was later confirmed in females, but surprisingly not in males [19], which is in contrast to another study including trained and untrained males [20].

In the present review we have collected and compared the previous studies in humans in which CS and $\dot{V}O_{2max}$ was measured before and after a training program with the purpose of characterizing the possible relationship between these two variables, and determine which factors that may influence this relationship. Such factors may include the training modality, age, gender, presence of metabolic or other diseases, initial fitness status, and methodological variations. Furthermore, the material allows for a direct comparison of absolute values of CS activity between the different studies with comparable study groups.

## Methods

### *Data sources and search profile*

A systematic search of literature on a bibliographical database PubMed published from 1983 to June 2013. We used the search profile: (citrate) AND synthase) AND human) AND skeletal) AND muscle) AND training) NOT electrical stimulation) NOT in-vitro) NOT rats.

### *Inclusion and exclusion*

We included all available studies in which CS activity in skeletal muscle (vastus lateralis) was measured as a marker for improved skeletal muscle oxidative capacity. We limited the search to human studies that included measurements of whole body oxygen uptake ($\dot{V}O_{2max}$) before and after a physical training intervention program. Studies were excluded if the subjects did not complete an incremental $\dot{V}O_{2max}$ test to exhaustion. Finally, cross-sectional studies and detraining studies were excluded (**Table 1** and **Figure 1**).

**Table 1.** Included studies

| Reference | | | Group characteristics | | | | | Intervention | | Aerobic adaptations | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Author | Year | Reference [##] | Group number | Group | Group characteristics | n | Gender | Training type | Total time trained (min) | Baseline $\dot{V}O_{2max}$ ($ml \cdot min^{-1} \cdot kg^{-1}$) | ΔCS activity (%) | $\Delta\dot{V}O_{2max}$ (%) |
| Allenberg et al. | 1988 | [64] | 1 | DI | Patients with type 2 diabetes | 7 | Males | ET | 2232 | N/A | 36 | 7 |
| Bakkman et al. | 2007 | [66] | 2 | CON | Healthy young untrained | 8 | Mixed | ET | 480 | 45 | 21 | 40 |
| Bangsbo et al. | 2010 | [67] | 3 | CON | Untrained running group | 25 | Females | ET | 1920 | 33 | 11 | 15 |
| | | | 4 | CON | Untrained football group | 25 | Females | ET | 1920 | 36 | 12 | 10 |
| Barnett et al. | 2004 | [51] | 5 | CON | Young healthy untrained | 16 | Males | HIT | 54 | N/A | 42 | 8 |
| Berthon et al. | 1995 | [68] | 6 | OLD | Healthy Elderly | 14 | Males | ET | 1440 | 35 | 46 | 6 |
| Blomstrand et al. | 2011 | [49] | 7 | CON | Healthy young sedentary | 5 | Mixed | ET | 1500 | 48 | 29 | 31 |
| | | | 8 | CON | Healthy young sedentary | 4 | Mixed | HIT | 1080 | 48 | 7 | 26 |
| | | | 9 | CON | Healthy young sedentary | 5 | Mixed | HIT | 1680 | 48 | 32 | 36 |
| Bruce et al. | 2004 | [5] | 10 | DI | T2DM patients | 6 | Males | ET | 1440 | 28 | 73 | 26 |
| | | | 11 | CON | Healthy control | 7 | Males | ET | 1440 | 31 | 85 | 18 |
| Bruce et al. | 2006 | [69] | 12 | OB | Obese | 9 | Mixed | ET | 2400 | 24 | 68 | 26 |
| Brønstad et al. | 2012 | [108] | 13 | DI | COPD patients | 12 | Males | HIT | 288 | 20 | 28 | -1 |
| Burgomaster et al. | 2005 | [34] | 14 | CON | Healthy young recreationally active | 16 | Males | HIT | 198 | 49 | 11 | 6 |
| Burgomaster et al. | 2008 | [48] | 15 | CON | Healthy young recreationally active | 10 | Mixed | HIT | 45 | 41 | 16 | 7 |
| | | | 16 | CON | Healthy young recreationally active | 10 | Mixed | ET | 1500 | 41 | 30 | 7 |
| Bylund et al. | 1977 | [4] | 17 | CON | Young healthy untrained | 20 | Males | ET | 5040 | N/A | 46 | 26 |
| Carter et al. | 2001 | [6] | 18 | CON | Healthy young active but untrained | 8 | Males | ET | 2100 | 42 | 40 | 17 |
| | | | 19 | CON | Healthy young active but untrained | 8 | Females | ET | 2100 | 32 | 43 | 24 |
| Charifi et al. | 2003 | [65] | 20 | OLD | Elderly healthy untrained | 11 | Males | ET | 2520 | 29 | 33 | 14 |
| Coggan et al. | 1992 | [71] | 21 | OLD | Elderly healthy untrained | 12 | Males | ET | 6864 | 27 | 29 | 24 |
| | | | 22 | OLD | Elderly healthy untrained | 11 | Males | ET | 6864 | 22 | 17 | 21 |
| Dawson et al. | 1998 | [9] | 23 | TR | Young fit | 9 | Males | HIT | N/A | 57 | -32 | 6 |
| Dubouchaud et al. | 2000 | [72] | 24 | CON | Healthy young sedentary | 9 | Males | ET | 3240 | 44 | 75 | 15 |
| Duscha et al. | 2012 | [43] | 25 | OLD | Obese healthy middle age/elderly | 40 | Mixed | ET | 32578 | 28 | 23 | 7 |
| | | | 26 | OLD | Obese healthy middle age/elderly | 47 | Mixed | ET | 17784 | 29 | 39 | 11 |
| | | | 27 | OLD | Obese healthy middle age/elderly | 41 | Mixed | ET | 33670 | 28 | 48 | 20 |
| Ferketich et al. | 1998 | [42] | 28 | OLD | Elderly | 24 | Females | ET | 1080 | 18 | 11 | 24 |
| Green et al. | 1992 | [103] | 29 | CON | Healthy young active but untrained | 9 | Males | ET | 720 | 55 | 5 | 2 |
| Green et al. | 1999 | [74] | 30 | CON | Yong healthy with low $\Delta\dot{V}O_{2max}$ | 7 | Males | ET | 120 | 41 | 21 | 9 |
| Green et al. | 2000 | [75] | 31 | CON | Healthy young sedentary | 10 | Males | HIT | 96 | N/A | -2 | -2 |

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Green et al. | 2009 | [76] | 32 | CON | Young healthy | 9 | Males | ET | 600 | 48 | 14 | 4 |
| | | | 33 | TR | Yong healthy with high $\Delta\dot{V}O_{2max}$ | 7 | Males | ET | 120 | 51 | 30 | 2 |
| Gurd et al. | 2010 | [77] | 34 | CON | Healthy young active but untrained | 9 | Mixed | HIT | 720 | 45 | 31 | 11 |
| Harmer et al. | 2008 | [78] | 35 | DI | Young T1DM | 8 | Mixed | HIT | 56 | N/A | 11 | -9 |
| | | | 36 | CON | Young healthy | 7 | Mixed | HIT | 56 | N/A | 42 | -3 |
| Heilbronn et al. | 2007 | [15] | 37 | OB | Insulin sensitive | 9 | Males | ET | 960 | 48 | 5 | 17 |
| | | | 38 | DI | Insulin resistant | 9 | Males | ET | 960 | 48 | 5 | 11 |
| Hiatt et al. | 1996 | [59] | 39 | DI | Intermittent claudication | 10 | Males | ET | 2160 | 15 | 10 | 17 |
| Houmard et al. | 1993 | [61] | 40 | CON | Sedentary healthy middle aged | 13 | Males | ET | 2888 | 30 | 69 | 21 |
| Howarth et al. | 2004 | [79] | 41 | CON | Young healthy M | 8 | Males | ET | 2100 | N/A | 32 | 6 |
| Iaia et al. | 2009 | [50] | 42 | TR | Young healthy trained | 17 | Males | HIT | 68 | 55 | -5 | -2 |
| Irving et al. | 2011 | [80] | 43 | CON | T2DM offspring | 8 | Mixed | ET+HIT | 945 | 26 | 11 | 0 |
| | | | 44 | CON | Healthy control | 8 | Mixed | ET+HIT | 945 | 27 | 23 | 0 |
| Jeppesen et al. | 2006 | [81] | 45 | DI | Patients with mtDNA mutations | 20 | Mixed | ET | 1500 | 26 | 66 | 27 |
| Jeppesen et al. | 2012 | [104] | 46 | CON | Healthy young sedentary | 8 | Males | ET | 1560 | 38 | 36 | 15 |
| | | | 47 | CON | Healthy matched subjects | 11 | Mixed | ET | 1500 | 34 | 65 | 21 |
| Kohn et al. | 2011 | [10] | 48 | TR | Young well trained | 18 | Males | HIT | 194,4 | 67 | -4 | 3 |
| Lange et al. | 2000 | [82] | 49 | DI | Healthy elderly Women | 8 | Females | HIT | 2160 | 22 | 35 | 17 |
| LeBlanc et al. | 2004 | [107] | 50 | CON | Young healthy | 8 | Males | ET | 2400 | N/A | 40 | 15 |
| Linossier et al. | 1997 | [11] | 51 | CON | Healthy young recreationally active | 8 | Males | HIT | 1800 | 52 | 7 | 3 |
| Luden et al. | 2011 | [83] | 52 | CON | Active young | 6 | Mixed | ET | N/A | 50 | 66 | 9 |
| MacDougall et al. | 1998 | [84] | 53 | CON | Healthy young recreationally active | 9 | Males | HIT | 84 | 51 | 25 | 3 |
| Mandroukas et al. | 1984 | [105] | 54 | CON | Obese | 14 | Females | ET | 1800 | N/A | 27 | 19 |
| Martin III et al. | 1989 | [85] | 55 | CON | Healthy young sedentary | 6 | Males | ET+HIT | 2520 | 46 | 40 | 20 |
| Masuda et al. | 2001 | [86] | 56 | CON | Healthy young sedentary | 7 | Males | ET | 1680 | 45 | 28 | 16 |
| McKenzie et al. | 2000 | [45] | 57 | CON | Healthy young sedentary | 6 | Males | ET+HIT | 1500 | 46 | 34 | 12 |
| | | | 58 | CON | Healthy young sedentary | 6 | Females | ET+HIT | 1500 | 38 | 27 | 18 |
| Messonier et al. | 2005 | [87] | 59 | CON | Untrained young | 8 | Mixed | ET | 2880 | 43 | 54 | 8 |
| Mogensen et al. | 2009 | [88] | 60 | DI | Type 2 diabetics | 12 | Males | ET | 625 | 27 | 58 | 11 |
| | | | 61 | DI | Obese | 11 | Males | ET | 625 | 29 | 37 | 15 |
| Moore et al. | 1987 | [21] | 62 | CON | Healthy young sedentary | 4 | Mixed | ET | 2310 | 45 | 59 | 22 |
| | | | 63 | CON | Trained healthy | 8 | Mixed | ET | 2310 | N/A | -13 | 1 |
| Murias et al. | 2011 | [53] | 64 | OLD | Active elderly | 7 | Males | ET | 1620 | 29 | 48 | 27 |
| | | | 65 | CON | Active young | 7 | Males | ET | 1620 | 49 | 67 | 16 |
| Ngo et al. | 2012 | [23] | 66 | OLD | Healthy elderly | 5 | Males | HIT | 3360 | 38 | 43 | 9 |

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Perry et al. | 2008 | [81] | 67 | CON | Healthy young recreationally active | 8 | Mixed | HIT | 720 | 45 | 26 | 9 |
| Perry et al. | 2010 | [90] | 68 | CON | Healthy young recreationally active | 9 | Males | HIT | 420 | N/A | 28 | 12 |
| Putman et al. | 1998 | [91] | 69 | CON | Healthy young recreationally active | 7 | Males | ET | 840 | 45 | 5 | 3 |
| Randers et al. | 2010 | [60] | 70 | CON | Young healthy | 10 | Males | ET | 4992 | 40 | 18 | 8 |
| Rud et al. | 2012 | [92] | 71 | CON | Healthy young sedentary | 8 | Mixed | ET | 1960 | N/A | 14 | 6 |
| Schantz et al. | 1983 | [28] | 72 | TR | Trained | 6 | Males | ET | 15840 | 61 | 0 | 0 |
| Sjödin et al. | 1982 | | 73 | CON | Healthy young sedentary | 8 | Males | ET | 280 | N/A | 11 | 2 |
| Slivka et al. | 2013 | [93] | 74 | CON | Young trained | 10 | Males | ET | N/A | N/A | 11 | 2 |
| Stannard et al. | 2010 | [93] | 75 | CON | Young healthy untrained | 7 | Mixed | ET | 1000 | N/A | 18 | 5 |
| | | | 76 | CON | Young healthy untrained | 7 | Mixed | ET | 1000 | N/A | 19 | 5 |
| Starritt et al. | 1999 | [95] | 77 | CON | Healthy young active but untrained | 7 | Mixed | ET | 600 | 44 | 26 | 9 |
| Svedenhag et al. | 1983 | [54] | 78 | CON | Healthy young sedentary | 8 | Males | ET | 1280 | 43 | 62 | 7 |
| Svedenhag et al. | 1983 | [96] | 79 | CON | Healthy young sedentary | 8 | Mixed | ET | 1280 | N/A | 75 | 7 |
| Talanian et al. | 2007 | [97] | 80 | CON | Recreational active | 8 | Females | HIT | 280 | 36 | 20 | 13 |
| Tarnopolsky et al. | 2007 | [44] | 81 | CON | Healthy young active but untrained | 5 | Males | ET | 2100 | 43 | 26 | 9 |
| | | | 82 | CON | Healthy young active but untrained | 7 | Females | ET | 2100 | 37 | 3 | 13 |
| Tiidus et al. | 1996 | [52] | 83 | CON | Healthy young sedentary | 7 | Males | ET | 840 | 48 | 25 | 12 |
| | | | 84 | CON | Healthy young sedentary | 6 | Females | ET | 840 | 37 | 50 | 22 |
| Tonkonogi et al. | 2000 | [98] | 85 | CON | Healthy young untrained | 8 | Mixed | ET | 960 | 39 | 47 | 24 |
| Trappe et al. | 2006 | [99] | 86 | CON | Recreational active | 7 | Mixed | ET | 5460 | 50 | 37 | 5 |
| Tynni-Lenné et al. | 1999 | [47] | 87 | DI | Patients with heart failure | 8 | Mixed | ET | 480 | 18 | 23 | 3 |
| | | | 88 | DI | Patients with heart failure | 8 | Mixed | ET | 480 | 16 | 45 | 19 |
| Vogiatzis et al. | 2005 | [7] | 89 | DI | COPD patients | 10 | Mixed | HIT | 1350 | N/A | 43 | 9 |
| | | | 90 | DI | COPD patients | 9 | Mixed | ET | 900 | N/A | 40 | 5 |
| Wibom et al. | 1992 | [100] | 91 | CON | Healthy young untrained | 9 | Males | ET | 864 | 44 | 43 | 10 |
| Yfanti et al. | 2010 | [101] | 92 | TR | Moderately trained | 10 | Males | ET+HIT | 5400 | 50 | 54 | 18 |
| | | | 93 | TR | Moderately trained | 11 | Males | ET+HIT | 5400 | 51 | 50 | 22 |
| Zoll et al. | 2005 | [102] | 94 | TR | Young healthy trained | 9 | Males | ET | 2013 | 64 | 1 | 5 |
| | | | 95 | TR | Young healthy trained | 6 | Males | ET | 2090 | 59 | -17 | 3 |
| Østergård et al. | 2005 | [106] | 96 | CON | Healthy untrained | 29 | Mixed | ET | 1350 | 38 | 25 | 14 |
| | | | 97 | CON | Healthy untrained | 19 | Mixed | ET | 1350 | 41 | 25 | 15 |

All studies and intervention groups included from search. Group column describes categorization in **Figure 5**: CON; Young healthy sedentary subjects, DI; studies investigating training in patients with various diseases, TR; Endurance trained subjects at inclusion. Group characteristics column: The group as described by the authors. Training type column: ET; The subjects performed endurance training, HIIT; The subjects performed high-intense interval training. Inclusion $\dot{V}O_{2max}$ column: N/A; not reported clearly in the study. Baseline $\dot{V}O_{2max}$: $\dot{V}O_{2max}$ reported before the intervention.

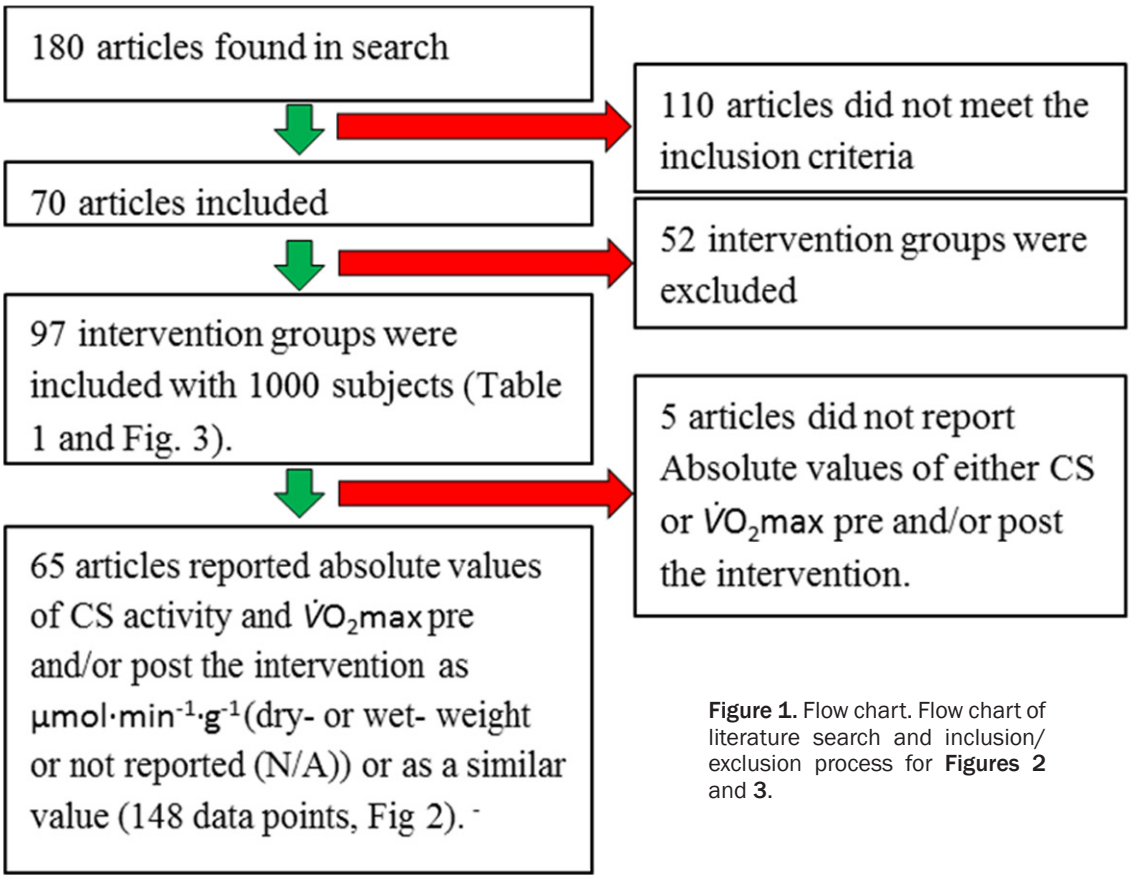


**Figure 1.** Flow chart. Flow chart of literature search and inclusion/exclusion process for **Figures 2** and **3**.

*Data extraction*

Two authors screened the retrieved articles and relevant studies were independently assessed. One author used a standardized form to extract data; a second author controlled the data for accuracy. Discrepancies were resolved by consensus or third-party adjudication. We constructed tables displaying: First authors, publication year, group characteristics, gender, number of subjects, $\dot{V}O_{2max}$ at inclusion, delta CS activity and delta $\dot{V}O_{2max}$.

The subjects were characterized as described in the study and the groups were primarily stratified according to men/females, young/elderly, trained/sedentary, healthy/disease (**Table 1**). If not defined in the article we defined elderly as age above 60 years and trained as a $\dot{V}O_{2max}$ above 55 and 50 ml $O_2$ min$^{-1}$·kg$^{-1}$ for men and women, respectively.

We wanted to study the isolated effect of HIIT and ET, therefore we excluded studies where detraining and resistance training was used [21-24], where spinal cord injuries were studied [25], electrical stimulation was used as stimulation [26], and studies where other muscles (deltoid or triceps brachii) were biopsied and analyzed [23, 27, 28].

Furthermore, we excluded a study if the main estimate for changes in aerobic capacity were $Watt_{max}$ [29-32], a time trial [33-35] or time to exhaustion [36]. This was done to allow a comparison of the relative improvement in $\dot{V}O_{2max}$ by using the same units for endurance performance.

Some studies only reported pre values of citrate synthase activity and/or $\dot{V}O_{2max}$ and hence it was not possible to calculate a relative change [37-41]. Furthermore, 5 studies reported values of CS activity that were more than a factor $10^3$ different from other studies, when the unit for CS activity was recalculated to the unit used in the present review, μmol·min$^{-1}$.g$^{-1}$. We assumed in those cases that the reported unit in the original article was erroneous, but accepted the reported value and included the

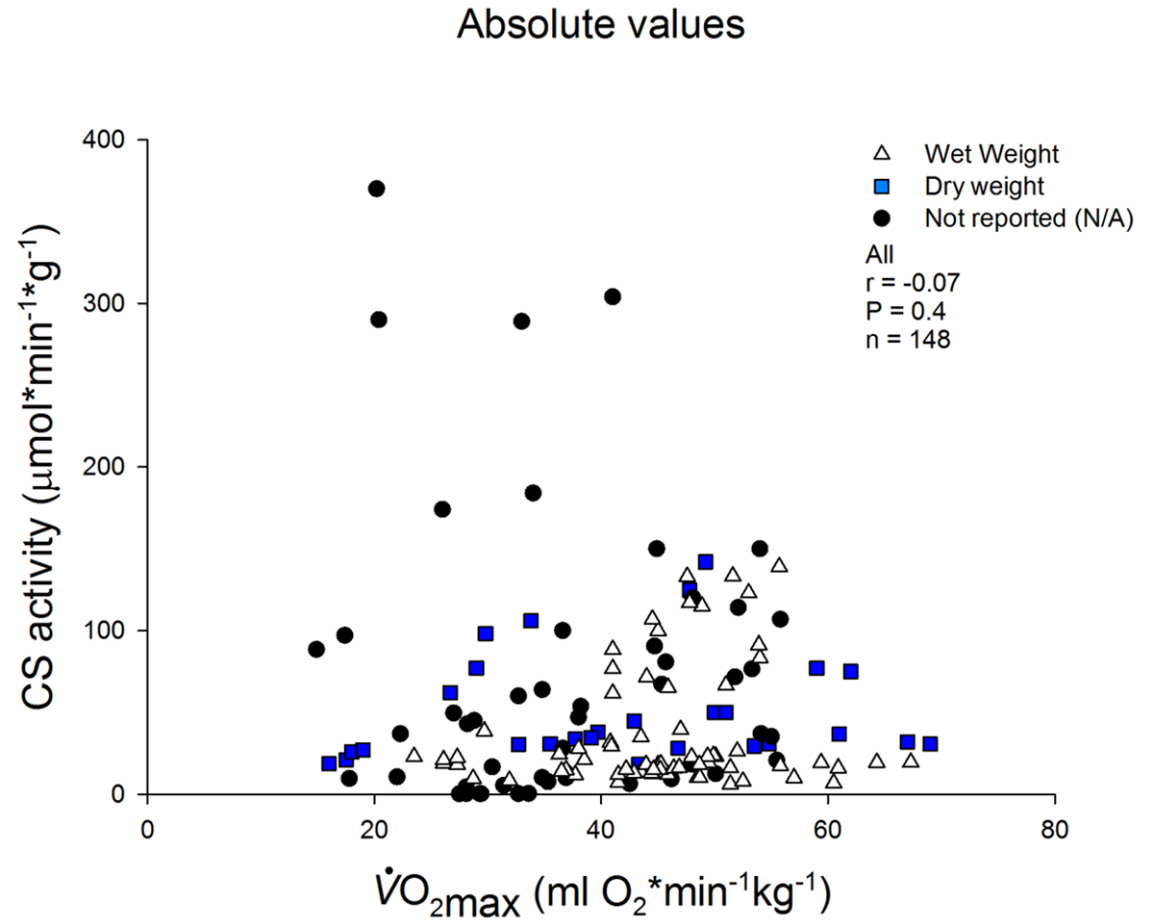


**Figure 2.** Absolute $\dot{V}O_{2max}$ and CS activity before and after interventions. 65 studies (n = 148 data points). The data points are divided by the sample preparation before analysis: wet (no preparation), dry (samples were freeze dried and dissected free of visible blood, fat and connective tissue) of not report (N/A, if the publication did not state clearly how the samples were prepared).

data in **Figure 2** [6, 42-45]. 5 studies only reported relative changes, and no absolute values of either CS or $\dot{V}O_{2max}$ pre and/or post the intervention, and these studies were not included in **Figure 2**. Two studies [46, 47] reported the same results from the same study and Gordon *et al.* was excluded.

Various terms describing a HIIT training program is used in the included publications (i.e. High Intense Training (HIT), High Intense Interval Training (HIIT), High Intensity Intermittent Exercise (HIIE), and Sprint Training (SIT)). For the purpose of the present review, all of these are termed High Intense Interval Training (HIIT).

*Statistics*

All statistical analyses were performed in Sigma Plot 12.5 (Systat software, Inc., San Jose, USA). The level of significance was set at $P < 0.05$. For correlations between different variables Pearson's product moment correlation coefficient ($r$) and corresponding $P$-value were obtained.

## Results

*Inclusion and exclusion*

The literature search identified 180 articles. 110 articles did not meet the inclusion criteria and were excluded. In the remaining 70 articles 149 intervention groups were identified. But 52 intervention groups did not meet the inclusion criteria and were excluded. The main reasons for exclusion were: the groups performed strength training, studied other muscle groups or were control groups. A total of 97 interven-

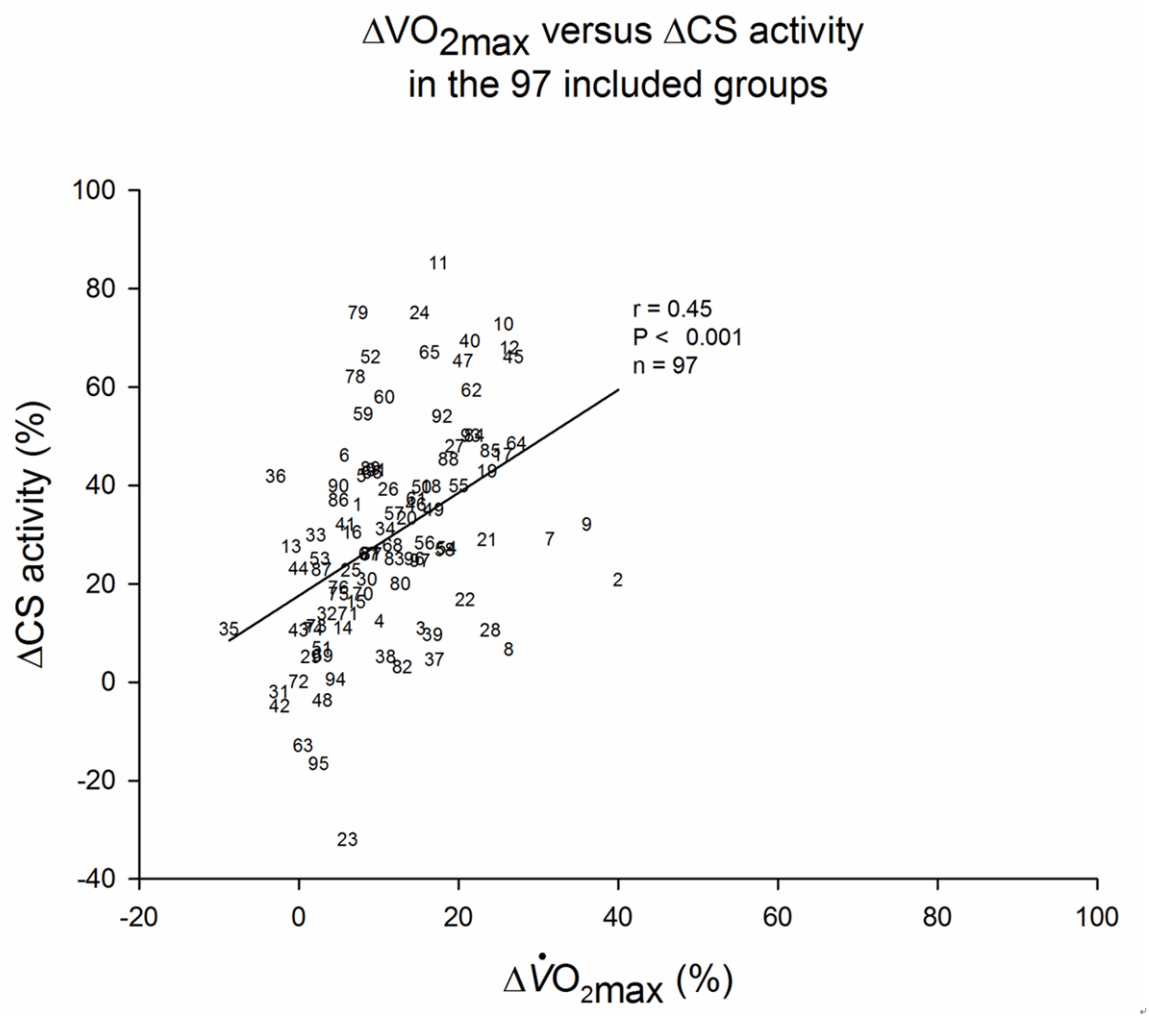


**Figure 3.** Relative changes in $\dot{V}O_{2max}$ and CS activity. The relative $\dot{V}O_{2max}$ and CS increase pre and post a training intervention in the 98 included groups. Number refers to the group number in **Table 1**.

tion groups including 1000 subjects were included in this review (**Table 1** and **Figure 1**).

*Absolute CS activity values*

There was no relationship between absolute measures of CS and $\dot{V}O_{2max}$ when we included all time points ($n$ = 148) from studies ($n$ = 65) that reported both CS activity as μmol· $min^{-1}$·g (wet or dry weight)$^{-1}$ and $\dot{V}O_{2max}$ $kg^{-1}$ ($r$ = -0.07, $P$ = 0.4, **Figure 2**). 12 studies including 28 study groups reported CS activity relative to dry weight (freeze dried and dissected free of visible connective tissue, lipids and blood). CS activity normalized to dry weight as an isolated factor did not correlate to $\dot{V}O_{2max}$ ($r$ = 0.11, $P$ = 0.60). Neither did the 33 studies with 68 groups that normalized CS activity to wet weight correlate to $\dot{V}O_{2max}$ when analysed alone ($r$ = 0.18, $P$ = 0.17). 20 studies (52 groups) did not report (N/A) clearly how the biopsies were treated prior to analysis, here there was no correlation between CS activity and $\dot{V}O_{2max}$ ($r$ = -0.14, $P$ = 0.33, **Figure 2**).

*Relative CS activity values*

The relative changes in $\dot{V}O_{2max}$ and CS activity in response to a training intervention in 97 intervention groups (**Table 1** and **Figure 3**) were significantly correlated ($r$ = 0.45, $P$ < 0.001). The equation for the trend line is: $\Delta CS = 1.1\ \Delta\dot{V}O_{2max} + 16.8$. The significant correlation was present also when all the included study groups were stratified according to training type (**Figure 4** and **Table 1**): ET ($r$ = 0.42, $n$ = 69, $P$ < 0.001), and combined ET and HIIT ($r$ = 0.81, $n$ = 7, $P$ < 0.05), but not with HIIT alone ($r$ = 0.24, $n$ = 21,

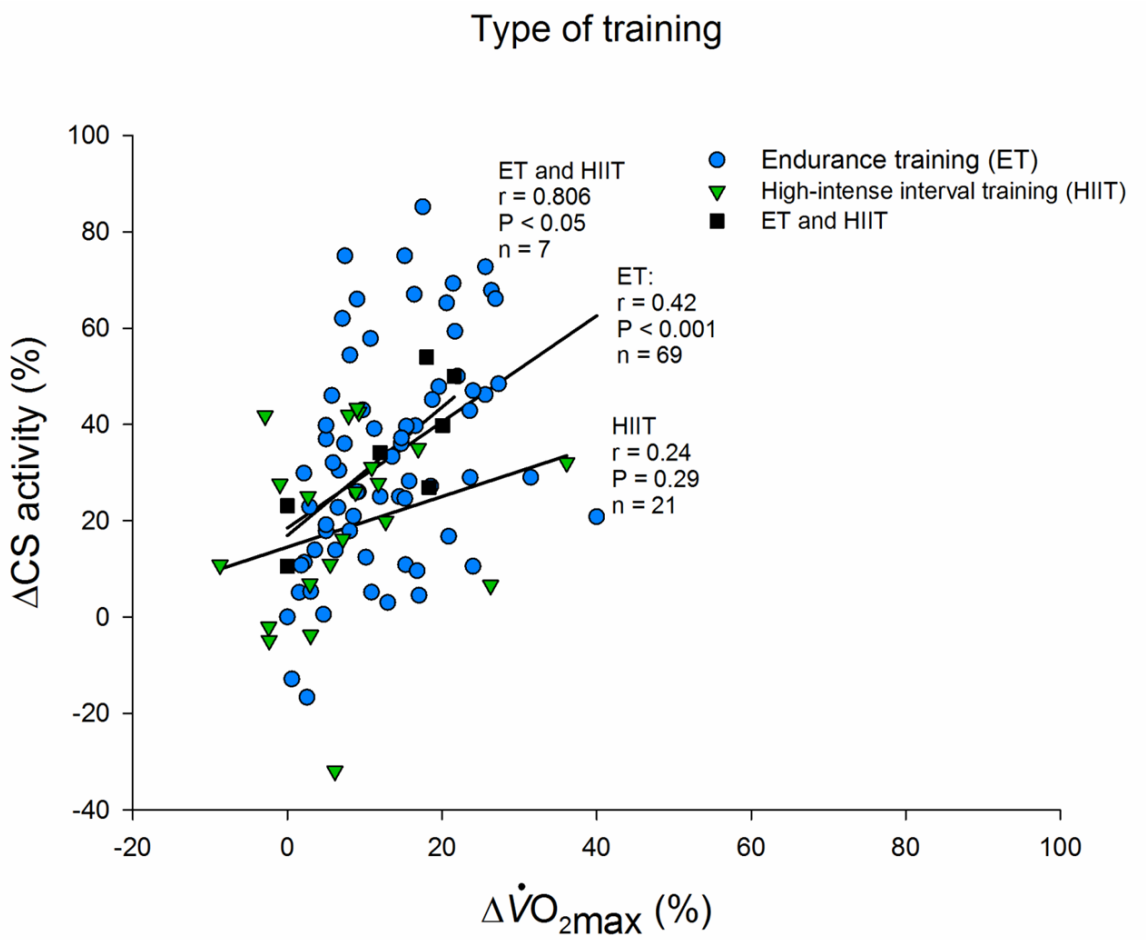


**Figure 4.** Type of training. All included groups from search expressed as the relative aerobic improvement and relative CS increase pre and post a training intervention divided by type of training used in the intervention: Endurance training, High-intensity interval training or a combination of Endurance training (ET) and High-intensity interval training (HIIT). The training type is divided by the description by the authors.

*P* = 0.29). Stratification according to inclusion background (**Figure 5** and **Table 1**) showed significant correlations in young sedentary subjects ($r$ = 0.35, $n$ = 63, $P$ < 0.05), endurance trained subjects ($r$ = 0.79, $n$ = 9, $P$ < 0.05), and in patients with various diseases and complications ($r$ = 0.67, $n$ = 14, $P$ < 0.05), but not in elderly subjects ($r$ = 0.20, $n$ = 10, $P$ = 0.57). By stratification according to gender (**Figure 6** and **Table 1**) only males correlated ($r$ = 0.63, $n$ = 52, $P$ < 0.001). In studies using females alone ($r$ = 0.57, $n$ = 10, $P$ = 0.08) or groups of mixed gender ($r$ = 0.31, $n$ = 35, $P$ = 0.07), there was only a tendency towards a correlation.

## Discussion

There is a clear positive and significant correlation between the relative change in $\dot{V}O_{2max}$ and in CS activity in response to physical training (**Figure 3**). There was almost a 1:1 relationship between the relative change in CS activity and change in $\dot{V}O_{2max}$. Thus, a ≈ 9% increase in CS activity may be expected from a 10% increase in $\dot{V}O_{2max}$. It is noteworthy that this relationship was not present when the correlation analysis was constrained to HIIT training or in elderly subjects alone. Oppositely, the relationship was intact when considering young sedentary subjects, trained subjects, and males alone. Likewise, both endurance training studies and studies combining HIIT and endurance training displayed a significant correlation between changes in $\dot{V}O_{2max}$ and CS activity. Absolute values of $\dot{V}O_{2max}$ and CS activity did not correlate, indicating that absolute measures of CS activity cannot be compared across studies and

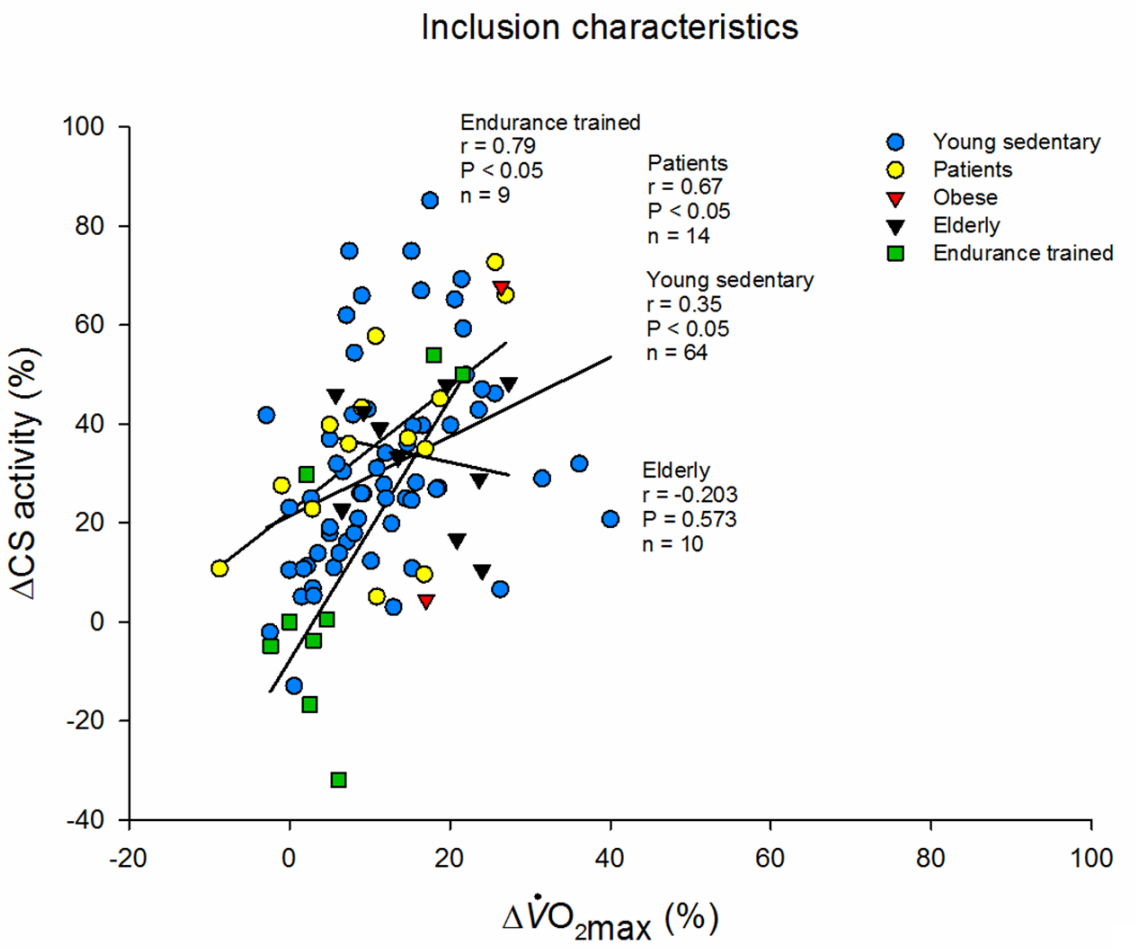


**Figure 5.** Subject background. All included groups from search expressed as the relative aerobic improvement and relative CS increase pre and post a training intervention divided the subject background included in the study: Young sedentary subjects, patients with various diseases, see **Table 1**, Obese subjects, Elderly subjects or endurance trained subjects. The characteristics are listed as described by the authors and can be seen in **Table 1**.

hence not be used for characterization of subject groups between different studies.

*Training type (ET and HIIT)*

One purpose of this review was to collect and analyze previously published studies in order to determine magnitudes and interrelationships in changes of $\dot{V}O_{2max}$ and CS in response to ET and HIIT. We found a positive and significant correlation between improvements in $\dot{V}O_{2max}$ and increases in CS activity in response to endurance training. This finding was not unexpected, but in contrast to this is, the lack of relationship between improvement in $\dot{V}O_{2max}$ and CS activity in response to HIIT was unexpected. The two forms of training elicited similar average improvements in $\dot{V}O_{2max}$ (ET: ≈ 13% and HIIT: ≈ 8%) but ET (≈ 33%) lead to higher improvement in CS activity compared to HIIT (≈ 19%). This underlines the major importance of cardiac performance for maximal oxygen uptake. Since CS activity in skeletal muscle is well correlated with mitochondrial volume in skeletal muscle [2, 3], the lower increase in CS activity with HIIT also indicate that mitochondrial biogenesis may not be stimulated at the same level as ET. In the studies where HIIT did not lead to an increase in CS activity, a significant increase in $\dot{V}O_{2max}$ was found in two [8, 9] of these five studies [7, 10, 11]. The differences in the CS response may be due a large variation in total training time ranging from 45 min [48] to 3360 min [23] and intensity ranging from 75-95% $HR_{max}$ [23] to 150 % $\Delta\dot{V}O_{2max}$ [49] in the included HIIT studies. Another factor is that it is

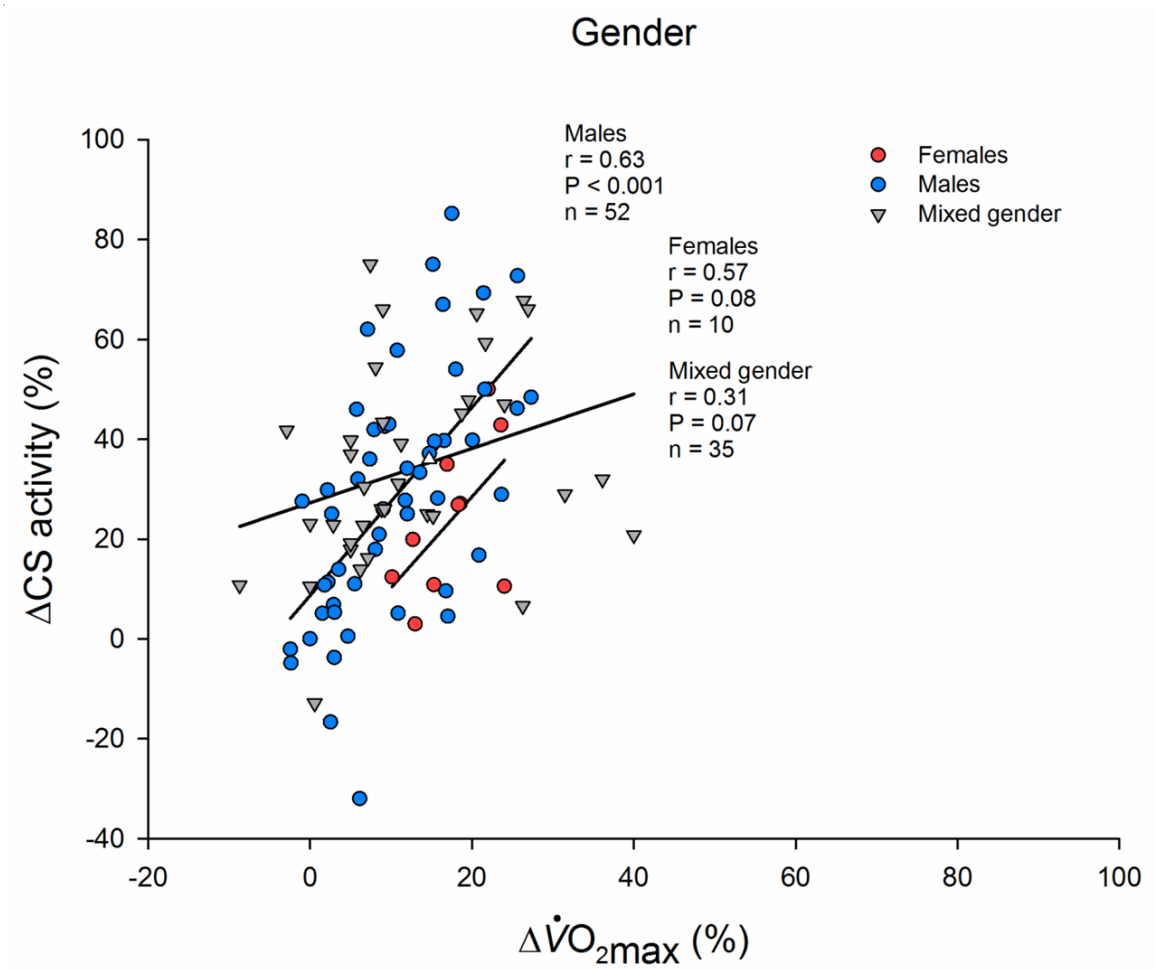


**Figure 6.** Gender. All included groups from search expressed as the relative aerobic improvement and relative CS increase pre and post a training intervention divided by the gender included in the study.

inherent in the nature of HIIT that the time spent training is less than that with endurance training (ET: ≈ 53 hr/study vs. HIIT: ≈ 12 hr/study in the included studies). The high intensity exercise for a short period may apparently be sufficient to elicit a cardiac adaptation (primarily an increase in maximal cardiac output), but not an adaptation of an important enzyme in the Krebs cycle in skeletal muscle.

On the other hand, the lack of significant relationship between ΔCS activity and $\Delta\dot{V}O_{2max}$ in the collective HIIT studies may also be due to three distinct studies (no 8, 9, and 23 in **Table 1**; the 3 triangles in **Figure 4** located most low-right) where disproportionate responses were seen. With exclusion of these three studies, a significant correlation is seen ($r$ = 0.48, $n$ = 18, $P$ < 0.05).

Some HIIT studies have been used to induce improvements in endurance performance in already highly trained athletes, measured as time to exhaustion or time trial [10, 50]. But these athletes did not have further increases in $\dot{V}O_{2max}$ or CS activity. It is possible that these athletes had already reached a plateau in the metabolic adaptations from the prior ET.

From the data it appears that a 8 wk. HIIT protocol with 3 training sessions per week each consisting of two to six 30 second sprint intervals was a highly time-efficient study [51]. This resulted in a 42% increase in CS activity with a total of 54 min. effective training [51]. Similar improvements in response to HIIT were shown in elderly subjects but after a longer HIIT training period [7]. The largest relative improvement (50-75%) in CS activity was seen in studies with endurance training [5, 21, 52-54]. These stud-

ies are all characterized by a high volume of total training and inclusion of subjects with a relatively low initial whole body $\dot{V}O_{2max}$. Even though it is highly speculative, it is possible that the nature of HIIT interventions is too short or extreme to allow mitochondrial biogenesis.

*Ageing*

The expected relationship between in improvements in $\dot{V}O_{2max}$ and CS activity was not observed in the studies ($n$ = 10) with elderly subjects (**Figure 5**). A 20 % decline in CS activity has been reported with age independent of lifestyle in some studies [14, 55], while others are inconclusive [56]. In contrast, other mitochondrial oxidative enzyme activities, for example the activity of complex I-IV are unaltered [14, 57]. Therefore, it is possible that adaptability in CS activity is altered with aging independently of changes in mitochondrial respiratory capacity, which has also been shown experimentally [57, 58].

A recent study by Duscha and colleagues reports a discrepancy between the relative improvement in CS activity and $\dot{V}O_{2max}$ in 3 groups (40-65 years) that performed different amount and intensity (low amount moderate-intensity, low amount-high intensity or high amount-high intensity training) of aerobic training (group # 25-27, **Table 1**). Only in the group that performed high amount-high intensity training ($r$ = 0.304, $n$ = 41) a positive correlation between relative $\dot{V}O_{2max}$ and CS activity was seen (group # 27, **Table 1**) [43, 58]. Thus, these findings indicate that in middle-aged and elderly a high amount-high intensity training program is necessary for improvement in both CS and $\Delta \dot{V}O_{2max}$.

*Gender*

We observed that only studies that included males alone showed significant correlation between $\dot{V}O_{2max}$ and CS activity. In studies ($n$ = 10) including women alone the relationship was only nearly significant ($P$ = 0.08), which is probably due to lack of statistical power. Is has been suggested [19] that transcriptional, translational, and/or post-translational regulation of CS is different between females and males at rest and immediately after acute exercise. However, this notion is not based on sound physiological considerations, and it remains to be proven.

*Methodological differences: dry or wet weight?*

There was no correlation between absolute values of $\dot{V}O_{2max}$ and CS activity in the included studies. The freeze-drying and dissection procedure of the muscle samples should have eliminated some variation due to contamination with non-muscle tissue/cells, but even in these samples, there was no correlation between the absolute values of $\dot{V}O_{2max}$ and CS. Although the measurements and analytical variation of $\dot{V}O_{2max}$ is well standardized across different laboratories, some day-to-day variation must be expected. Less standardized is the biochemical analysis CS activity. This analysis requires relatively small muscle biopsies, approximately 2-3 mg d.w. corresponding to 10-15 mg w.w. In the authors laboratory CS activity is measured spectrophotometrically as described by Srere [17] at 37°C. The assay has a low inter- and intra assay variation. We find an inter-assay variation of 4.2% in the low range (27 ± 1 (mean ± SD) μmol·min$^{-1}$·g (d.w.)$^{-1}$) and 0.8% in the high range (613 ± 5 μmol·min$^{-1}$·g (d.w.)$^{-1}$) and an intra-assay variation of 2.5% in the low range (28 ± 1 μmol·min$^{-1}$·g (d.w.)$^{-1}$) and 4.8% in the high range (589 ± 5 μmol·min$^{-1}$·g protein (d.w.)$^{-1}$) (unpublished data). These are lower than was has been reported for analyses in non-freeze dried and un-dissected tissue (4.9% [34], 5.4% [49] and 7.7% [6] in the low range of CS activity). This speaks for analyzing on dissected tissue. Another major factor for variation in absolute values of CS activity is the analytical temperature (usually 25-37°C), which is, unfortunately, not always reported. Increased activity with 37°C compared to 25°C must be expected. Finally, it would be possible to correct data for blood contamination with e.g. creatine correction or other methods, but this is very seldom reported.

Five studies recruited a non-training control group [43, 50, 59-61]. In these groups no statistical change in $\Delta\dot{V}O_{2max}$ or ΔCS activity were reported. However, the ΔCS activity reported varies from 10% decrease (NS) [60] to 14% increase (NS) [61]. This indicates that some physiological time related variation should be expected when measuring.

*Responders and non-responders*

Despite a positive correlation between $\Delta\dot{V}O_{2max}$ and ΔCS activity there is a considerable varia-

tion in the relationship (**Figure 3**). We have suggested that training regimes, subject background or methodological variation contributes to this. However, it has to be considered that there is a significant inter-subject variation in training induced adaptations in $\dot{V}O_{2max}$, which increases the variation [62, 63]. The molecular mechanisms underlying the variation in response to exercise training are still poorly understood, but it is possible that also adaptations in CS activity may be individual. A close inspection of **Table 1** and **Figure 3** reveals that group 23, 31, 42, 48, 63, and 95 reported a negative ΔCS activity.

*Limitations*

CS activity and $\dot{V}O_{2max}$ are not always reported both pre and post training. This excluded a large number of studies, and thus removes valuable information. Furthermore, we decided to remove measures of aerobic capacity that was not $\dot{V}O_{2max}$, but measured as e.g. time trial or $Watt_{max}$. There is a large variation in how these tests are conducted, which increases the variation in the results between studies. Cross-sectional studies were not taken into consideration. Inclusion of the many cross-sectional studies in the literature may have provided additional information on the absolute values of CS activity across various studies (**Figure 2**). Finally, the lack of relationship in females and elderly subjects may be due to a low number of included studies studying these groups, which then may have provided a false negative result.

## Conclusions

Most factors (young sedentary or trained subjects, males, ET and combined ET and HIIT) showed a positive and significant linear relationship between $\Delta\dot{V}O_{2max}$ and ΔCS activity. This was not the case in publications studying HIIT, females and elderly subjects. CS activity as a marker of mitochondrial density should be used with care in studies using very short term HIIT. The lack of relationship in the females and maybe also in the elderly is most likely a statistical power problem. Finally, a large methodological variation in the analysis of CS activity between laboratories is probably the major reason for a lack of significant relationship in absolute values in $\dot{V}O_{2max}$ and CS activity.

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## Disclosure of conflict of interest

The authors declare that they have no conflict of interest.

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