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Systematic Review

The Effect of High-Intensity Interval Training on Mitochondrial-Associated Indices in Overweight and Obese Adults: A Systematic Review and Meta-Analysis

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Abstract

Background: Obesity is a significant health problem with an increasing incidence, causing a low-grade systemic inflammatory state and being implicated in various chronic diseases. Moreover, obesity has been shown to cause mitochondrial dysfunction through oxidative stress and inflammation, eventually affecting energy metabolism. However, high-intensity interval training (HIIT) can improve mitochondrial efficiency through exercise-induced mitochondrial adaptations. This systematic review and meta-analysis aims to examine the potential effects of HIIT on mitochondrial-associated indices in obese and overweight adults. **Methods**: PubMed, Scopus, and Web of Science databases were searched. **Results**: Twenty-eight eligible studies were included, involving 530 participants. HIIT was found to significantly improve the activity of citrate synthase (CS), cytochrome C (COX-IV), beta-hydroxyacyl CoA-dehydrogenase (β-HAD), Complexes I-V as well as VO_2max in overweight and obese individuals, whereas no significant changes were shown in PGC-1α and SIRT1. Interestingly, subgroup analyses revealed that CS, COX-IV, β-HAD, and Complexes I-V activity exhibited a significant improvement only in the healthy subgroup. **Conclusions**: Overall, HIIT can be utilized to enhance mitochondrial-associated indices in overweight and obese individuals. However, this improvement may be health status dependent.

Keywords: citrate synthase; cytochrome c; Complex I–V; mitochondria; biogenesis; obesity; physical activity; exercise

1. Introduction

Obesity, a result of the imbalance between energy intake and energy expenditure, has been widely characterized as a global pandemic. As reported by the World Health Organization (WHO), worldwide obesity has nearly tripled since 1975. In 2016, over 1.9 billion (39%) adults aged 18 years and older were overweight. Of these, over 650 (13%) million were obese [1]. Furthermore, one billion people are estimated to be obese by 2030, as predicted in the World Obesity Atlas 2022, according to the World Obesity Federation [2]. Obesity reflects an excessive accumulation of adipose tissue, which is considered an active organ playing a role in the body's homeostasis processes and metabolism [3]. Moreover, an increased infiltration of obese people's adipose tissue by immune cells has been observed, which in turn promotes the development of a mild pro-inflammatory environment both locally and systemically. Chronic inflammation may lead to type 2 diabetes (T2D) and insulin resistance, cardiovascular diseases, fatty liver disease, asthma, neoplasia, as well as mitochondrial dysfunction [3,4].

Mitochondria are responsible for the generation of adenosine triphosphate (ATP), the primary energy source for the function of cells and tissues [5]. During the ATP synthesis process through the tricarboxylic acid cycle and oxidative phosphorylation, reactive oxygen species (ROS) are released [6]. When the ROS production exceeds the antioxidant defense systems, oxidative stress is initiated [7]. Moreover, the excessive intake of nutrients in obesity increases mitochondrial ROS production, thus leading to oxidative stress. This stress, in turn, can cause mitochondrial function impairment, reduced mitochondrial biogenesis, and decreased rate of β-oxidation [8]. Additionally, increased ROS production results in damage to mitochondrial and nuclear nucleic acids, lipid membranes and proteins, and especially enzymes of the mitochondrial respiratory chain [9]. Thus, a vicious cycle is created by oxidative stress, inflammation, and mitochondrial dysfunction.

Certain diseases may affect mitochondrial function and biogenesis, leading to significant variations in cellular energy production and overall health. Neurodegenerative and cardiovascular diseases have been associated with impaired mitochondrial function, contributing to the progressive degeneration of neurons and impacting cardiac muscle function and energy metabolism accordingly [10,11]. Additionally, hypertension has been linked to mitochondrial

dysfunction in vascular cells [12]. In turn, damage to these structures results in their dysfunction and has been linked to a wide range of diseases [13,14]. Understanding the unique effects of these diseases on mitochondrial biology is essential for elucidating their underlying mechanisms and exploring potential therapeutic interventions.

Peroxisome proliferator-activated receptor-gamma coactivator 1-alpha (PGC-1α) is a protein often called the "master regulator" of mitochondrial biogenesis. One of the ways it is activated is through deacetylation by the silent information regulator 1 (SIRT1). It also regulates the expression of mitochondrial antioxidant genes [15]. In situations characterized by increased caloric intake, such as metabolic syndrome, the levels of PGC-1α decrease, which can result in a reduction in mitochondrial biogenesis. This situation promotes ROS accumulation and reduces muscle oxidative capacity and aerobic capacity [16].

Physical activity and/or exercise have increasingly been suggested as effective strategies to manage obesity. Indeed, the value and importance of exercise in health has been highlighted since ancient times. Hippocrates from Kos, Greece, the "father of medicine", was the first recorded physician to recognize the necessity of physical exercise and prescribing written exercise for a patient suffering from consumption [17]. The main benefits of exercise are increased maximal oxygen uptake and increased fat oxidation in healthy individuals and patients with metabolic diseases [18,19]. The WHO recommends an equivalent combination of regular moderate- and vigorous-intensity physical activity (exercise) for at least 150 minutes per week [20].

Many forms of exercise training can be used based on the exercise characteristics, i.e., frequency, intensity, duration, and type of exercise [21]. High-intensity interval training (HIIT) consists of repeated bouts of short ($<$45 seconds) or long (2–4 minutes) duration high-intensity exercise ($\geq$80% of maximal heart rate), interspersed with brief recovery periods [22]. HIIT, in addition to its time-efficiency over other forms of exercise training, appears superior to moderate intensity continuous training (MICT) in improving cardiorespiratory fitness, mitochondrial function, and consequently physical performance [21,23–27]. More specifically, HIIT has been shown to promote mitochondrial adaptations through the up-regulation of key mitochondrial enzymes and changes in mitochondrial morphology and content [28]. Indeed, increases in the activity of citrate synthase (CS), PGC-1α, beta-hydroxyacyl CoA-dehydrogenase (β-HAD), cytochrome C (COX-IV) and respiratory chain complexes (Complex I–V) have been observed following HIIT [28,29]. As intense and interval training has been shown to promote mitochondrial biogenesis through changes in PGC-1α, as well as mitochondrial protein synthesis, HIIT appears to be a promising training exercise for obese people [30]. However, there has not been a systematic review and synthesis of the existing quantitative evidence for the effects of HIIT on mitochondrial-associated indices in overweight and obese people. Therefore, this systematic review and meta-analysis aims to examine whether HIIT exercise improves mitochondrial-associated indices in overweight and obese individuals, with the secondary objective of examining and comparing the differences in HIIT-induced alterations of mitochondrial-associated indices between healthy individuals and those with a medical history.

2. Materials and Methods

This systematic review was structured according to the Preferred Reporting Items of Systematic Reviews and Meta-Analyses (PRISMA) guidelines [31]. The review protocol was registered with the international prospective register for systematic reviews (PROSPERO) database (registration number: CRD42022381060) [32].

2.1 Search Strategy and Selection Process

A systematic search was conducted by two independent researchers (SH and SMD) through the electronic databases PubMed, Scopus, and Web of Science, from the day of their inception until March 2022. Using a combination of keywords relative to the three main axes of the study (HIIT exercise, obesity, and mitochondrial-associated indices) provided us with a full range of relevant literature. Variations were made to the algorithm according to the specificities of each electronic database. A forward snowballing technique (searching the reference list of the eligible studies to identify potential article omissions that could also be eligible) was used by researcher IAK to ensure literature saturation. Limitations were applied to exclude animal trials and publications in languages other than English. Two independent reviewers (SH and IAK) selected the eligible studies. Reviewer disagreements were resolved through a referee investigator (PCD).

2.2 Inclusion and Exclusion Criteria

Predetermined criteria based on the PICOS (population, intervention, comparator, outcomes, and study design) framework guided the screening process, which was performed by researchers SH and IAK [33]. As a population, we defined obese or overweight people as those who were included in studies with a body mass index (BMI) population mean value of $\geq$27 kg/m^2, based on the recent association of a BMI above 27 kg/m^2 with poor/fair health-related quality of life compared with good and very good/excellent health-related quality of life from an observational web-based study with over 17,000 participants [34]. Both men and women were included, without age or comorbidity limits. The intervention consisted of high-intensity interval training (HIIT) for $\geq$6 sessions and $\geq$2 weeks of intensity $\geq$80% of maximal heart rate. RCTs, CT, and single-arm (pre vs. post) studies were included. As a control situation, we accepted either a control group or baseline mea-

surements compared with post-intervention measurements. The outcomes we focused on were: (1) Complex I–V, (2) COX-IV, (3) CS, (4) β-HAD, (5) SIRT1, (6) PGC-1α and (7) mitochondrial density, morphology, volume, content, size. Studies were excluded if they did not meet the above criteria.

We intended to synthesize evidence and evaluate heterogeneity from the available literature comprehensively. Therefore, we had no restrictions in our inclusion criteria and accepted outcomes of various expression levels (protein level, enzyme activity, gene expression, and respiratory capacity). Additionally, both muscle and plasma samples were included, considering the established excellent correlation between muscle and serum PGC-1α levels [15]. The meta-analysis aimed to investigate mitochondrial responses to training at the cellular and molecular level across various tissues. The inclusion of both tissue types in our analysis was driven by the desire to comprehensively understand the systemic impact of training on mitochondrial-associated indices while maintaining the practicality, robustness, and heterogeneity of our findings.

2.3 Risk of Bias Assessment

For the eligible RCTs, we used the updated Risk of Bias 2 (RoB2) Cochrane library tool [35]. For the quasi RCT, CT as well as the single arm eligible studies, we used the Research Triangle Institute Item Bank (RTI-IB) tool [36], to assess the risk of bias. Two independent reviewers (SH and IAK) assessed the risk of bias in eligible studies, while a referee investigator (PCD) resolved any disagreements between the two independent reviewers.

2.4 Data Extraction and Quality Assessment

Two independent reviewers (SH and SMD) extracted data from the eligible studies as follows: (a) first author surname and year of publication, (b) methodological design, (c) participants' anthropometric characteristics (i.e., age, gender), (d) BMI, (e) exercise intervention, (f) main outcome. The extracted data can be found in the **Supplementary Material** (**Supplementary Table 1**). Additional data was requested via email to the corresponding author in two cases. Firstly, we requested and subsequently obtained subjects' weight and height data for a study referring to an overweight population without determining BMI [37]. However, we were unable to access data regarding the mean age and sex of subjects in another study [38]. Despite this, both studies were ultimately included in our meta-analysis. If the outcome data were only provided in graph form, we utilized an online plot digitizer to extract the necessary values [39].

2.5 Data Synthesis

A summarized narrative data synthesis was adopted for eligible studies that did not provide numerical data to be used for a meta-analysis. For studies suitable for meta-analysis, a random-effect model was used to account for heterogeneity due to differences in study populations, HIIT interventions, and study duration. All meta-analyses were conducted using the Review Manager software (RevMan) v.5.4.1, 2020 (The Cochrane Collaboration, Copenhagen, Denmark) [40]. We used an inverse variance, a continuous method, to calculate mean differences either between a HIIT intervention group and a control group or between baseline and post-intervention values of the intervention group. In cases where the calculation units were different for the variable involved in a meta-analysis, a standardized mean difference (SMD) approach was used [33]. We analyzed the following variables: CS, COX-IV, β-HAD, PGC-1α, SIRT1, mitochondrial respiration Complex I–V, and VO_2max. We used subgroup analyses to identify differences between participants: (a) with BMI <30 kg/m^2 and those with BMI >30 kg/m^2, and (b) healthy vs. disease. To differentiate between the two health subgroups, we relied on the health reports provided by the individual studies, as indicated by their respective authors. Individuals without any comorbidities, except for obesity, were categorized in the healthy group, whereas individuals with reported comorbidities were grouped into the disease group. Where pertinent, we converted the standard error into standard deviation (SD), using the equation:

$$SD = standard\ error * \sqrt{n} \quad \text{[33].}$$

As non-parametric and parametric data cannot be mixed in a meta-analysis, we converted the means and SD of non-parametric data into parametric data, when necessary, using well-established equations [41]:

$$mean = exp\ (mean + \tfrac{SD2}{2});$$

$$\text{SD} = \sqrt{exp\ exp\ (SD2) - 1)\ \ exp(2mean + SD2)}$$

[41].

2.6 Evidence of Effectiveness

We evaluated the quality of evidence of each meta-analysis via the Grading of Recommendations Assessment, Development and Evaluation (GRADE) analysis [33,42].

3. Results

Reporting information is shown in the relevant PRISMA checklist in the **Supplementary Material** (**Supplementary Fig. 1**) [31].

3.1 Results of Searching and Selection Processes

Of the 2338 retrieved publications, 529 were duplicates, and 1695 did not meet the prespecified eligibility criteria and were excluded based on title and abstract. Additionally, ten publications that did not fulfill the inclusion criteria were also excluded after careful consideration of their full text. The screening of the reference lists of the eligible publications revealed one more eligible paper. Ultimately, 28 studies were included in this systematic review, as shown in the PRISMA 2020 flow diagram (**Supplementary Fig. 1**) [31].

3.2 Characteristics of the Included Studies and Risk of Bias Assessment Outcomes

The risk of bias assessment outcomes is shown in the **Supplementary Material** (**Supplementary Table 2** and **Supplementary Fig. 2**).

The studies included in this systematic review were published between the years 2011 and 2022, showing how recent and, at the same time, rapidly growing the literature on this topic is. The population of all eligible studies included 530 overweight or obese participants and 51 healthy controls. Five studies were randomized controlled trials (RCTs) [15,43–46], 19 studies were single-arm trials [37,38,47–63], two studies were parallel-group trials [64,65], and two were non-randomized parallel controlled trials (CTs) [66,67].

Regarding the eligible RCTs [15,43–46], one indicated a high risk of bias [43], and four showed some concerns [15,44–46] of risk of bias in the randomization process. In the intervention assignment, four revealed some concerns [43–46] and one indicated a low risk of bias [15]. Regarding intervention adherence, the risk of bias was low for all five studies [15,43–46]. For missing data, one RCT showed a high risk of bias [45], and the remaining four RCTs showed a low risk of bias [15,43,44,46]. Finally, for the outcome, all five RCTs demonstrated a low risk of bias [15,43–46]. For the reported results, two demonstrated a low risk of bias [15,45], and three showed some concerns about the risk of bias [43,44,46].

For selection bias in the CTs and single arm design studies, the risk was not applicable for 12 [37,38,47–52,54,56,59,62], low for seven [53,57,58,60,63–65], high for one [67] and three indicated some concerns [55,61,66]. For performance bias, two studies showed some concerns [56,59], and the other 21 showed a low risk of bias [37,38,47–55,57,58,60–67]. For detection bias, all 23 studies showed a low risk of bias [37,38,47–67]. For attrition bias, eight studies indicated a low risk [53,55,61,63–67], and 15 showed a not applicable risk of bias [37,38,47–52,54,56–60,62]. For selective outcome, eight studies showed low risk [38,55–60,67], and 15 revealed some concerns about risk of bias [37,47–54,61–66]. For confounding bias, all 23 studies displayed some concerns about the risk of bias [37,38,47–67].

3.3 Narrative Data Synthesis Results

A narrative data synthesis was adopted for the eligible studies that did not provide sufficient data for a meta-analysis. In particular, mitochondrial volume density, mitochondrial size, and mitochondrial number were calculated from only one study, which showed an increase after the intervention [64]. Furthermore, a single study provided results regarding the mitochondrial membrane potential of isolated neutrophils, which also displayed an increase after the exercise intervention [52].

3.4 Results of Meta-Analyses

The primary outcomes for the meta-analyses appear in Table 1.

3.4.1 Citrate Synthase

The meta-analysis for CS was performed with data obtained from 17 studies [37,38,43,44,47–51,53,55–58,60,62,66], which were expressed in protein content [56] or activity [37,38,43,44,47–51,53,55,57,58,60,62,66]. It revealed a statistically significant positive effect of HIIT on CS activity in the overweight and obese population ($p < 0.00001$) (Fig. 1, **Supplementary Figs. 3,4**). There were no significant differences in SMDs between BMI subgroups ($p = 0.48$) (**Supplementary Fig. 3**). However, when subjects were categorized by health status, a statistically significant increase in CS was found in healthy subjects ($p < 0.00001$), whereas those with a background of disease showed only a non-significant tendency for improvement ($p = 0.06$) (Fig. 1).

3.4.2 COX-IV

The meta-analysis for COX-IV was conducted using data from 10 studies [37,46,51,54,56,57,61,64,65,67], which were expressed in terms of protein content [46,51,56,57,61,65], protein density [67], protein intensity [54], fluorescence intensity [64] and flux [37]. It showed a statistically significant positive effect of HIIT on COX-IV activity in the overweight and obese population ($p < 0.00001$) (Fig. 2, **Supplementary Figs. 5, 6**). There were no significant differences in SMDs between BMI subgroups ($p = 0.41$) (**Supplementary Fig. 5**). The healthy status subgroup showed a significant increase in COX-IV ($p < 0.00001$) in contrast to the subgroup with elevated CVD risk ($p > 0.05$) (Fig. 2).

3.4.3 β-HAD

The meta-analysis for β-HAD was performed using data obtained from 11 studies [37,43,44,47,51,55,57,58,61,62,66], which were expressed in terms of protein content [43,61] and activity [37,44,47,51,55,57,58,62,66]. It revealed a statistically significant positive effect of HIIT on β-HAD activity in the overweight and obese population ($p < 0.0001$) (**Supplementary Figs. 7,8,9**). No significant differences in SMDs were found between BMI subgroups ($p = 0.66$) (**Supplementary Fig. 7**). However, when subjects were categorized by health status, a statistically significant increase in β-HAD was found in healthy subjects ($p < 0.00001$), whereas those with a history of disease did not show a significant increase ($p > 0.05$) (**Supplementary Fig. 8**).

3.4.4 PGC-1α

The meta-analysis for PGC-1α was performed with data obtained from six studies [15,43,45,51,56,61], which were expressed in terms of protein content [45,51,56,61,64]

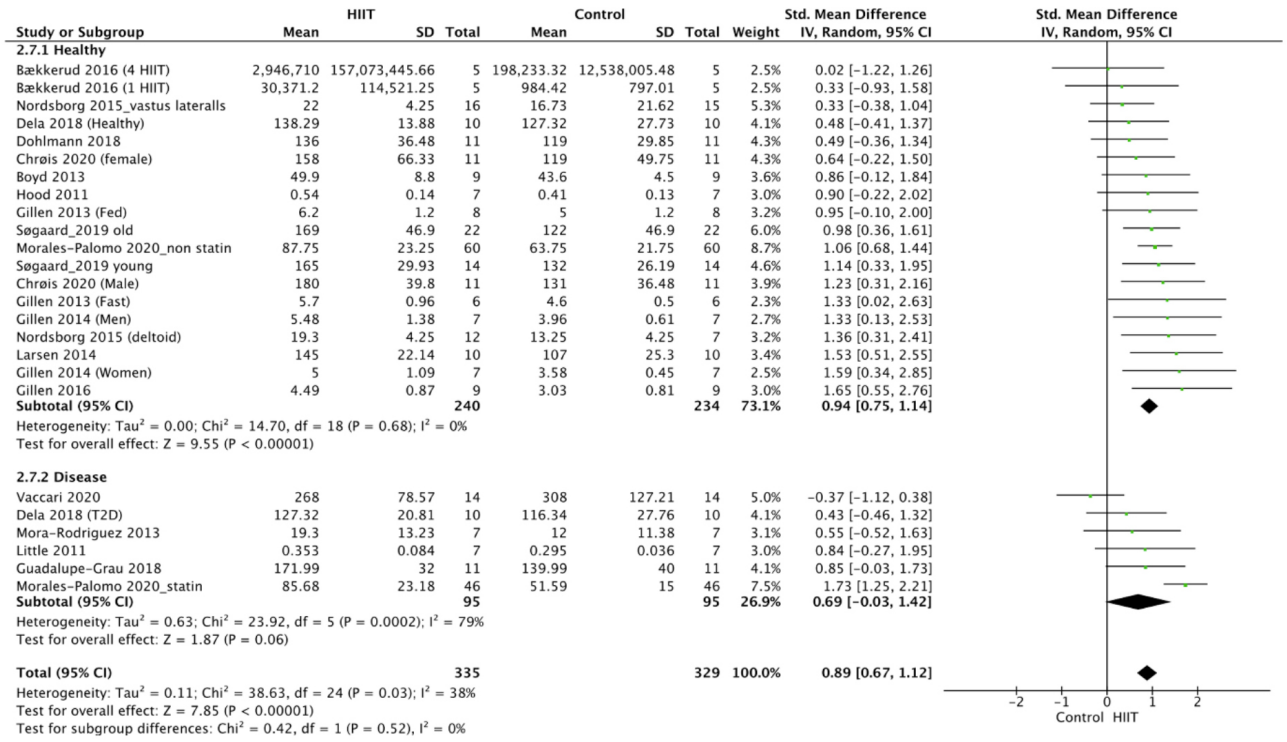

Fig. 1. Forest plot of the effect of HIIT on CS (subgroup analysis for health status). SD, standard deviation; 95% CI, 95% confidence interval.

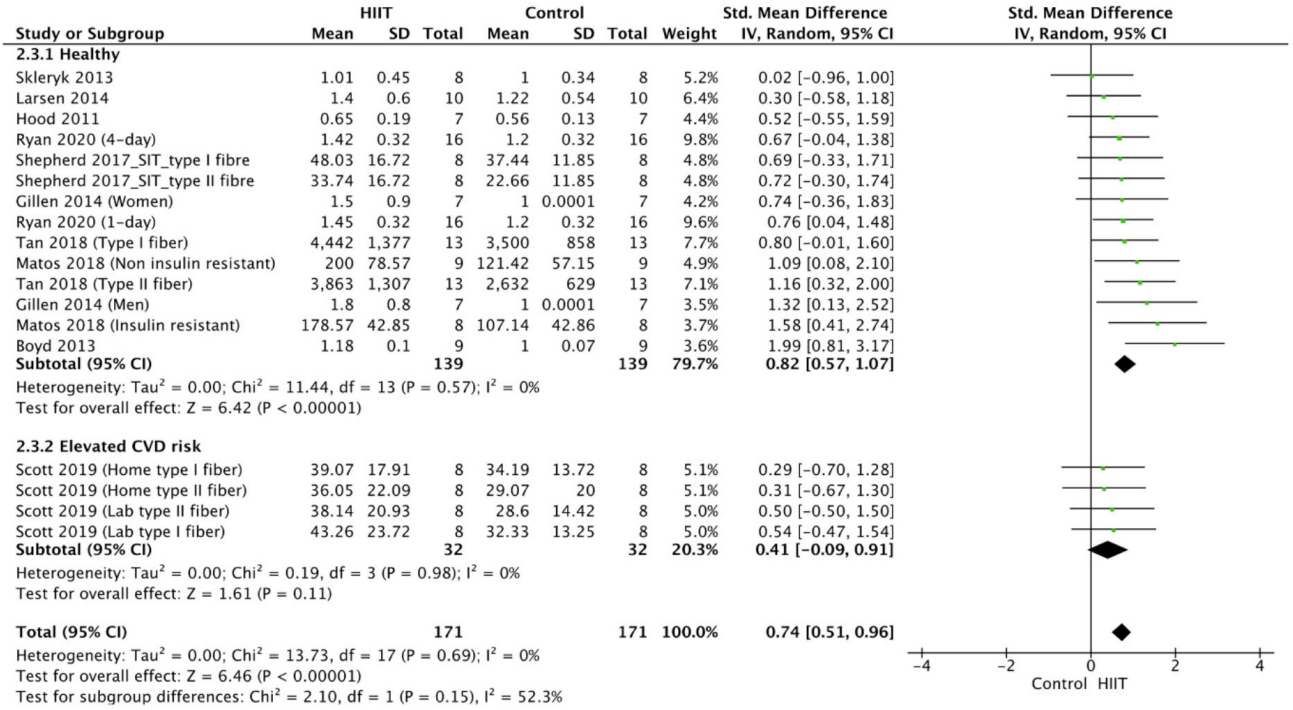

Fig. 2. Forest plot of the effect of HIIT on COX-IV (subgroup analysis for health status). SD, standard deviation; 95% CI, 95% confidence interval.

and gene expression [43] and revealed no significant effect of HIIT on PGC-1α activity in the overweight and obese population ($p > 0.05$) (**Supplementary Figs. 10,11**). However, the subgroup of overweight subjects (BMI <30 kg/m^2) showed a statistically significant increase in PGC-1α ($p < 0.0003$). In comparison, the obese subgroup (BMI

Table 1. Summary of meta-analyses and sub-group analyses results.

Meta-analysis	N (HIIT/control)	SMD [95% CI]	Heterogeneity (I^2)	Z score (p)	Subgroup difference
CS (overall)	335/329	0.89 [0.67, 1.12]	38%	7.85 ($p < 0.00001$)	-
CS (BMI <30 kg/m^2)	65/59	1.01 [0.61, 1.41]	5%	4.94 ($p < 0.00001$)	$p = 0.48$
CS (BMI >30 kg/m^2)	270/270	0.84 [0.57, 1.10]	47%	6.09 ($p < 0.00001$)	
CS (healthy)	240/234	0.94 [0.75, 1.14]	0%	9.55 ($p < 0.00001$	$p = 0.52$
CS (disease)	95/95	0.69 [–0.03, 1.42]	79%	1.87 ($p = 0.52$)	
COX-IV (overall)	171/171	0.74 [0.51, 0.96]	0%	6.46 ($p < 0.00001$)	-
COX-IV (BMI <30 kg/m^2)	49/49	0.89 [0.47, 1.31]	0%	4.13 ($p < 0.0001$)	$p = 0.41$
COX-IV (BMI >30 kg/m^2)	122/122	0.68 [0.41, 0.94]	0%	5.03 ($p < 0.00001$)	
COX-IV (healthy)	139/139	0.82 [0.57, 1.07]	0%	6.42 ($p < 0.00001$)	$p = 0.15$
COX-IV (disease)	32/32	0.41 [–0.09, 0.91]	0%	1.61 ($p = 0.11$)	
β-HAD (overall)	276/266	0.67 [0.37, 0.98]	61%	4.30 ($p < 0.0001$)	-
β-HAD (BMI <30 kg/m^2)	56/50	0.58 [0.19, 0.98]	0%	2.89 ($p = 0.004$)	$p = 0.66$
β-HAD (BMI >30 kg/m^2)	220/216	0.71 [0.30, 1.13]	73%	3.36 ($p = 0.0008$)	
β-HAD (healthy)	209/199	0.52 [0.32, 0.72]	0%	5.05 ($p < 0.00001$)	$p = 0.57$
β-HAD (disease)	67/67	0.89 [–0.39, 2.17]	89%	1.37 ($p = 0.17$)	
PGC-1α (overall)	96/95	0.51 [–0.03, 1.05]	68%	1.86 ($p = 0.06$)	-
PGC-1α (BMI <30 kg/m^2)	38/38	0.89 [0.41, 1.37]	0%	3.62 ($p = 0.0003$)	$p = 0.17$
PGC-1α (BMI >30 kg/m^2)	58/57	0.25 [–0.54, 1.04]	75%	0.61 ($p = 0.54$)	
PGC-1α (healthy)	86/85	0.59 [0.01, 1.18]	69%	1.98 ($p = 0.05$)	$p = 0.17$
PGC-1α (disease)	10/10	–0.15 [–0.03, 1.05]	N.A.	0.32 ($p = 0.75$)	
SIRT1 (overall)	60/59	0.81 [–0.13, 1.76]	82%	1.69 ($p = 0.09$)	-
SIRT1 (BMI <30 kg/m^2)	31/31	0.78 [–0.31, 1.88]	75%	1.40 ($p = 0.16$)	$p = 0.89$
SIRT1 (BMI >30 kg/m^2)	29/28	0.94 [–0.98, 2.86]	89%	0.96 ($p = 0.34$)	
Complex I (overall)	118/117	0.69 [0.21, 1.17]	66%	2.84 ($p = 0.005$)	-
Complex I (BMI <30 kg/m^2)	8/8	–0.52 [–1.53, 0.48]	N.A.	1.03 ($p = 0.31$)	$p = 0.02$
Complex I (BMI >30 kg/m^2)	110/109	0.80 [0.33, 1.27]	61%	3.34 ($p = 0.0008$)	
Complex I (healthy)	101/100	0.70 [0.15, 1.25]	69%	2.51 ($p = 0.28$)	$p = 0.90$
Complex I (disease)	17/17	0.62 [–0.50, 1.75]	58%	1.08 ($p = 0.28$)	
Complex II (overall)	103/102	0.38 [0.10, 0.66]	0%	2.64 ($p = 0.008$)	-
Complex II (BMI <30 kg/m^2)	8/8	–0.19 [–1.18, 0.79]	N.A.	0.38 ($p = 0.70$)	$p = 0.24$
Complex II (BMI >30 kg/m^2)	95/94	0.43 [0.14, 0.72]	0%	2.64 ($p = 0.004$)	
Complex II (healthy)	86/85	0.38 [0.07, 0.68]	0%	2.43 ($p = 0.02$)	$p = 0.98$
Complex II (disease)	17/17	0.39 [–0.38, 1.15]	18%	0.99 ($p = 0.32$)	
Complex III (overall)	87/88	0.79 [0.41, 1.18]	31%	4.03 ($p < 0.0001$)	-
Complex III (BMI <30 kg/m^2)	8/8	0.59 [–0.41, 1.60]	N.A.	1.15 ($p = 0.25$)	$p = 0.69$
Complex III (BMI >30 kg/m^2)	79/80	0.82 [0.38, 1.25]	40%	3.68 ($p = 0.0002$)	
Complex III (healthy)	70/71	0.87 [0.47, 1.27]	21%	4.28 ($p < 0.0001$)	$p = 0.64$
Complex III (disease)	17/17	0.57 [–0.64, 1.78]	63%	0.92 ($p = 0.36$)	
Complex IV (overall)	87/88	0.65 [0.07, 1.23]	68%	2.21 ($p = 0.03$)	-
Complex IV (BMI <30 kg/m^2)	8/8	–0.92 [–1.197, 0.12]	N.A.	1.73 ($p = 0.08$)	$p = 0.003$
Complex IV (BMI >30 kg/m^2)	79/80	0.84 [0.34, 1.33]	53%	3.31 ($p = 0.0009$)	
Complex IV (healthy)	70/71	0.57 [–0.03, 1.16]	65%	1.86 ($p = 0.06$)	$p = 0.61$
Complex IV (disease)	17/17	1.23 [–1.27, 3.72]	87%	0.96 ($p = 0.34$)	
Complex V (overall)	82/81	0.43 [0.11, 0.74]	0%	2.67 ($p = 0.008$)	-
Complex V (BMI <30 kg/m^2)	10/8	–0.10 [–1.03, 0.83]	N.A.	0.21 ($p = 0.83$)	$p = 0.24$
Complex V (BMI >30 kg/m^2)	72/73	0.50 [0.16, 0.83]	0%	2.91 ($p = 0.004$)	
Complex V (healthy)	72/71	0.49 [0.15, 0.82]	0%	2.84 ($p = 0.005$)	$p = 0.33$
Complex V (disease)	10/10	0.02 [–0.86, 0.90]	N.A.	0.04 ($p = 0.96$)	
VO_2max (overall)	678/485	3.14 [2.30, 3.99]	78%	7.30 ($p < 0.00001$)	-
VO_2max (BMI <30 kg/m^2)	87/87	3.93 [2.28, 5.57]	61%	4.68 ($p < 0.00001$)	$p = 0.27$
VO_2max (BMI >30 kg/m^2)	591/398	2.86 [1.88, 3.83]	70%	5.75 ($p < 0.00001$)	
VO_2max (healthy)	344/343	3.23 [2.22, 4.24]	82%	6.26 ($p < 0.00001$)	$p = 0.54$
VO_2max (disease)	334/142	2.78 [1.75, 3.81]	0%	5.29 ($p < 0.00001$)	

N.A., not applicable.

>30 kg/m^2) did not show a significant change ($p > 0.05$) (**Supplementary Fig. 10**). When subjects were categorized by health status, a statistically significant increase in PGC-1α was found in healthy subjects ($p = 0.05$). In contrast, those with a background of disease did not show a significant increase ($p > 0.05$) (**Supplementary Fig. 11**).

3.4.5 SIRT1

The meta-analysis for SIRT1 was performed with data obtained from four studies [15,45,51,65] and was expressed in terms of protein content [15,45,51,65]. The analysis of the data revealed no significant effect of HIIT on SIRT1 activity in the overweight and obese population ($p > 0.05$) (**Supplementary Fig. 12**). No significant difference in SMDs was found between BMI subgroups ($p = 0.89$) (**Supplementary Fig. 12**).

3.4.6 Complex I

The meta-analysis for Complex I was performed with data obtained from eight studies [38,43,46,49–51,62,63], which were expressed in terms of protein content [43,46,49–51,63] and mitochondrial respiratory capacity [38,62]. It showed a statistically significant positive effect of HIIT on Complex I in the overweight and obese population ($p < 0.005$) (**Supplementary Figs. 13,14**). The test applied to BMI subgroup differences showed a statistically significant difference in favor of obese (BMI >30 kg/m^2) ($p < 0.005$) over the overweight (BMI <30 kg/m^2) ($p > 0.05$) subgroup (Chi^2 = 5.50, I^2 = 81.8%, $p < 0.05$) (**Supplementary Fig. 13**). Additionally, the healthy status subgroup showed a significant increase in Complex I ($p = 0.01$) in contrast to the subgroup with a background of disease ($p > 0.05$) (**Supplementary Fig. 14**).

3.4.7 Complex II

The meta-analysis for Complex II was performed with data obtained from seven studies [38,43,46,49,50,63,65] which were expressed in terms of protein content [43,46,49,50,63,65] and mitochondrial respiratory capacity [38]. It revealed a statistically significant positive effect of HIIT on Complex II in the overweight and obese population ($p < 0.005$) (**Supplementary Figs. 15,16**). No significant differences in SMDs were found based on BMI or health status categories ($p > 0.05$) (**Supplementary Figs. 15,16**). However, obese subjects (BMI >30 kg/m^2) showed a significant increase in Complex II ($p < 0.005$) in contrast to overweight subjects (BMI <30 kg/m^2) ($p > 0.05$) (**Supplementary Fig. 15**). Additionally, the healthy status subgroup showed a significant increase in Complex II ($p < 0.05$) in contrast to the subgroup with a background of disease ($p > 0.05$) (**Supplementary Fig. 16**).

3.4.8 Complex III

The meta-analysis for Complex III was conducted with data obtained from five studies [43,46,49,50,63], which were expressed in terms of protein content [43,46,49,50,63] and revealed a statistically significant positive effect of HIIT on Complex III in the overweight and obese population ($p < 0.0001$) (**Supplementary Figs. 17,18**). No significant differences in SMDs were found between BMI or health status categories ($p = 0.69$ and $p = 0.64$, respectively) (**Supplementary Figs. 17,18**). However, obese subjects (BMI >30 kg/m^2) showed a significant increase in Complex III ($p < 0.0005$) in contrast to overweight subjects (BMI <30 kg/m^2) ($p > 0.05$), (**Supplementary Fig. 17**). Additionally, the healthy status subgroup showed a significant increase in Complex III ($p < 0.0001$) in contrast to the subgroup with a background of disease ($p > 0.05$), (**Supplementary Fig. 18**).

3.4.9 Complex IV

The meta-analysis for Complex IV was performed with data obtained from five studies [43,46,49,50,63], which were expressed in terms of protein content [43,46,49,50,63] and revealed a statistically significant positive effect of HIIT on Complex IV in the overweight and obese population ($p < 0.05$) (**Supplementary Figs. 19,20**). The test applied to BMI subgroup differences showed a statistically significant preponderance of obese (BMI >30 kg/m^2) ($p < 0.005$) over the overweight (BMI <30 kg/m^2) ($p > 0.05$) subgroup (Chi^2 = 8.87, I^2 = 88.7%, $p < 0.005$) (**Supplementary Fig. 19**). There were no significant differences in SMDs in the health status subgroup analysis ($p = 0.61$) (**Supplementary Fig. 20**).

3.4.10 Complex V

The meta-analysis for Complex V was performed with data obtained from four studies [43,46,50,63], which were expressed in terms of protein content [43,46,49,50,63] and revealed a statistically significant positive effect of HIIT on Complex V in the overweight and obese population ($p < 0.05$) (**Supplementary Figs. 21,22**). No significant differences in SMDs were found between BMI or health status categories ($p = 0.24$ and $p = 0.33$, respectively) (**Supplementary Figs. 21,22**). However, obese subjects (BMI >30 kg/m^2) showed a significant increase in the Complex V ($p < 0.005$) in contrast to overweight subjects (BMI <30 kg/m^2) who did not show a significant change ($p > 0.05$) (**Supplementary Fig. 21**). Additionally, the healthy status subgroup showed a significant increase in Complex V ($p = 0.005$) in contrast to the subgroup with a background of disease ($p > 0.05$) (**Supplementary Fig. 22**).

3.4.11 VO_2max

The meta-analysis for VO_2max was conducted with data obtained from 24 studies [15,37,38,43,45–48,50–55,58–67], which showed a statistically significant positive effect of HIIT on VO_2max in the overweight and obese population ($p < 0.00001$) (**Supplementary Figs. 23,24,25**).

Table 2. GRADE analysis outcomes.

Outcomes	No. of participants (studies/entries)	Quality of the evidence (GRADE)	Relative effect (95% CI)
HIIT on citrate synthase vs. control	664 (25 studies/entries)	⊕◯◯◯ due to imprecision	SMD = 0.89 (0.67–1.12)
HIIT on cytochrome c vs. control	342 (18 studies/entries)	⊕⊕◯◯ due to methodological design of the studies	SMD = 0.74 (0.51–0.96)
HIIT on β-HAD vs. control	542 (18 studies/entries)	⊕⊕◯◯ due to methodological design of the studies	SMD = 0.67 (0.37–0.98)
HIIT on PGC-1α vs. control	191 (10 studies/entries)	⊕⊕◯◯ due to imprecision	SMD = 0.51 [(–0.03)–(1.05)]
HIIT on Complex I vs. control	235 (12 studies/entries)	⊕◯◯◯ due to imprecision	SMD = 0.69 (0.21–1.17)
HIIT on Complex II vs. control	205 (10 studies/entries)	⊕⊕◯◯ due to methodological design of the studies	SMD = 0.38 (0.10–0.66)
HIIT on Complex III vs. control	175 (8 studies/entries)	⊕⊕◯◯ due to imprecision	SMD = 0.79 (0.41–1.18)
HIIT on Complex IV vs. control	175 (8 studies/entries)	⊕⊕◯◯ due to imprecision	SMD = 0.65 (0.07–1.23)
HIIT on Complex V vs. control	163 (7 studies/entries)	⊕⊕⊕◯ due to methodological design of the studies	SMD = 0.43 (0.11–0.74)
HIIT on VO_2max vs. control	1172 (34 studies/entries)	⊕⊕⊕◯ due to inconsistency	SMD = 3.14 (2.30–3.99)

CI, confidence interval; SMD, standardized mean difference; ⊕⊕⊕◯, Moderate; ⊕⊕◯◯, Low; ⊕◯◯◯, Very Low.

There were no significant differences in SMDs between BMI and health status subgroups (p = 0.27 and p = 0.54, respectively) (**Supplementary Figs. 23,24**).

3.5 Grade Analysis Outcomes

Our GRADE analysis outcomes appear in Table 2, while the detailed evaluation of its components can be found in the **Supplementary Material** (**Supplementary Table 3**). The meta-analysis outcomes of the effects of HIIT on Complex V vs. control and HIIT on VO_2max vs. control of overweight and obese participants showed a moderate quality of evidence. The meta-analyses of the effects of HIIT on CS, COX-IV, β-HAD, PGC-1α, Complex I, Complex II, Complex III, and Complex IV showed a low quality of evidence.

4. Discussion

4.1 Summary of Main Findings

The meta-analyses showed that HIIT can positively affect several mitochondrial-associated indices in overweight and obese individuals. Specifically, statistically significant improvements were observed in CS, COX-IV, β-HAD, Complex I, II, III, IV, and V activities, as well as in VO_2max. Despite observing a trend towards increased enzyme activity, there were no statistically significant effects of HIIT on PGC-1α and SIRT1. Further analyses of the data revealed interesting outcomes regarding subgroups based on BMI or health status of the participants.

For BMI, two main subgroups were investigated: BMI $<$30 kg/m^2 (overweight group) and BMI $>$30 kg/m^2 (obese group). Statistically significant subgroup differences in standardized mean differences (SMDs) based on BMI were recorded for Complex I and Complex IV activity, where a more favorable effect of HIIT was found in the obese subgroup compared with the overweight subgroup. Additionally, the results observed for Complexes II, III, and V showed a trend for the obese subgroup to have a greater influence on the enzymes' activity. However, these results cannot be considered reliable as the BMI $<$30 kg/m^2 subgroup only contained one study. In contrast, the overweight subgroup showed a greater influence on PGC-1α activity. Furthermore, there were no statistically significant differences in SMDs based on BMI subgroups for CS, COX-IV, β-HAD, VO_2max, and SIRT1.

In terms of health status, subjects were classified into two main groups: the healthy group and the disease group, which comprised subjects with a history of disease. Even though no statistically significant subgroup differences in SMDs were observed based on health status, a trend in favor of the healthy subgroup exists with respect to the effect of HIIT on CS, COX-IV, β-HAD, PGC-1α, and Complexes I, II, III, and V. However, these results should be interpreted with caution due to two reasons: firstly, except for Complex V and VO_2max, GRADE analysis revealed a low and very low level of quality of evidence, and secondly, the subgroup analysis for disease only contained one study. Regarding SIRT1, Complex IV, VO_2max, and health status aspects, no statistically significant differences were found, no substantial differences were observed, or it was not possible to extract outcomes with the available data.

4.2 Completeness and Applicability of Evidence

For the primary outcomes, there was sufficient evidence to assess, through the meta-analysis, the effect of HIIT on CS (17/28 included studies—60%), β-HAD (11/28 included studies—39%), COX-IV (10/28 included studies—35%), and, to a lesser extent Complex I (8/28 included studies—28%), Complex II (8/28 included studies—28%), PGC-1α (6/28 included studies—21%), Complex III (5/28 included studies—17%), Complex IV (5/28 included studies—17%), SIRT1 (4/28 included studies—14%) and Complex V (4/28 included studies—14%). For the secondary outcome, the evidence was sufficient in order to examine the effect of HIIT on VO_2max (24/28 included studies—85%). However, some of the outcomes (CS, COX-IV, β-HAD, PGC-1α, Complex I–IV) demonstrated low-quality evidence and thus should be treated with caution.

Our findings are consistent with previous research on the positive effects of HIIT on mitochondrial biogenesis and enzyme regulation in the general population [68–70]. In addition, previous findings have demonstrated the feasibility and beneficial effects of HIIT, particularly on overweight and obese populations with or without comorbidities. In those studies, improvements in cardiorespiratory function, including VO_2max, systolic blood pressure, body composition, lipid metabolism markers, and insulin resistance, were observed [23,71–74].

Based on the various markers we examined, our data suggest that HIIT can improve mitochondrial-associated indices in overweight/obese individuals. In particular, exercise volume and intensity are important to improve mitochondrial respiratory function and mitochondrial content, and HIIT can combine both exercise volume and intensity advantageously [69,75,76]. Furthermore, the reduced effect of HIIT on the mitochondrial parameters in the disease subgroup raises reasonable questions regarding potential contributing factors. These could be the impact of underlying diseases, the disease's severity and duration, and the treatment or medications received. In a meta-analysis by Morales-Palomo *et al*. [66] 2020, participants had metabolic syndrome and were undergoing chronic statin therapy. Statins have been reported to exert various effects on mitochondria, including reductions in coenzyme Q10 levels, inhibition of respiratory chain complexes, induction of mitochondrial apoptosis, dysregulation of Ca^{2+} metabolism, and altered expression of carnitine palmitoyltransferase-2, leading to a decrease in maximal mitochondrial respiration [43,77]. Furthermore, in the Dela *et al*. [43] 2019 study, participants were T2D patients with a number of them undergoing treatment with glucose-lowering agents, antihypertensive agents, or statin medication. As mentioned, due to the negative impact of statins on mitochondrial function, they were paused one-week prior to the experiment. However, glucose-lowering and antihypertensive agents were only paused on the day of the experiment. In another three studies where participants were undergoing antihypertensive or glucose-lowering medications, participants were instructed not to adjust their medications [48,49,67]. Consequently, the use of medications may attenuate the mitochondrial benefits derived from HIIT. However, both the underlying pathological background and medication intake collectively represent a chronic pathological condition, thus contributing to the disease subgroup context under investigation in our comparative analysis and highlighting the distinct mode of effect of HIIT in the healthy and the disease subgroups.

We also observed an analogous relationship between the increase in mitochondrial biogenesis and increase in VO_2max. VO_2max can be indirectly related to mitochondrial respiratory capacity [78,79]. Moreover, light has been shed in the last few years on the relationship between obesity and mitochondrial dysfunction [7]. Mitochondrial dysfunction is highly implicated in diseases such as metabolic syndrome, T2D, cardiovascular disease, and neurodegenerative diseases [10–12,80,81]. It is evident that, especially in populations that are overweight/obese or at high risk of becoming obese, implementing modalities like HIIT to improve mitochondrial function and cardiorespiratory fitness is of utmost importance to decrease the likelihood of disease in the forthcoming years.

4.3 Strengths and Potential Biases in the Review Process and Disagreement with Previous Systematic Reviews

The strengths of our systematic review include: (a) the use of suitable algorithms and standardized indexing terms during the search process to enabled the retrieval of publications that used alternative keywords to describe the same concept, (b) we conducted searching and screening processes, assessed the risk of bias, and extracted data in a thorough way, without excluding studies based on publication date, (c) for the evaluation of the quality of our meta-analyses, GRADE analysis was utilized, (d) to the best of the author's knowledge, no other systematic review has examined the effect of HIIT on mitochondrial-associated indices in overweight/obese individuals, and (e) the subgroup analysis comparing individuals with excess body weight alone and individuals with excess body weight and a disease factor revealed differences in the effectiveness of HIIT on mitochondrial-associated indices.

The present systematic review also has certain limitations, including: (a) lack of expert supervision for some HIIT exercise interventions, (b) the studies included were restricted to the English language, (c) some of our primary outcomes were assessed with low-quality evidence, (d) with the exception of three studies [47,48,66] the intervention period lasted from 2–12 weeks, (e) the complexity of assessing the diverse effects of certain diseases on mitochondrial biology, along with their implications for exercise-induced adaptations and the influence on mitochondrial-associated indices, and (f) the studies that utilized serum samples instead of skeletal muscle may not be directly comparable, as the assessment of comparability is based on one study.

4.4 Statement on the Significant Deviations Methods from the Published Protocol

We report no significant deviations from the published protocol.

5. Conclusions

The present data demonstrate that HIIT has the potential to significantly improve mitochondrial-associated indices in overweight and obese populations, leading to an overall improvement in health status, provided that certain conditions are met. Furthermore, it appears that, for overweight/obese individuals with an additional disease factor, HIIT is less effective in improving mitochondrial-

associated indices than their counterparts without diseases. However, a larger sample size of overweight/obese patients with comorbidities and better quality of evidence is needed to verify this observation. Overall, the findings of this systematic review and meta-analysis offer valuable insights into the positive effects of HIIT as a strategy for improving energy metabolism in overweight and obese populations. Nevertheless, more interventional RCTs are needed, especially of longer duration, investigating the effect of HIIT on mitochondrial-associated indices not only in overweight and obese individuals but also in other clinical populations, with or without comorbidities in order to reveal possible disease dependent HIIT-induced beneficial adaptations.

Availability of Data and Materials

All data included in this study are available upon request by contact with the corresponding author.

Author Contributions

Conceptualization, SH, SMD, IAK and AP; methodology, SH, PCD, CC and AP; software, PCD; validation, SH and PCD; formal analysis, PCD; investigation, SH, SMD, IAK; resources, SH and PCD; data curation SH, SMD, IAK, and AP; writing—original draft preparation, SH, SMD, IAK and PCD; writing—review and editing, SH, SMD, IAK, PCD, CC and AP. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

Not applicable.

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Conflict of Interest

The authors declare no conflict of interest.

Supplementary Material

Supplementary material associated with this article can be found, in the online version, at https://doi.org/10.31083/j.fbl2811281.

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