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REVIEW ARTICLE



Effect of high intensity interval training on body composition in women before and after menopause: a meta-analysis

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Abstract

High-intensity interval training (HIIT) is a stimulating modality for reducing body weight and adipose tissue. The purpose of this meta-analysis was to assess the efficacy of HIIT in reducing weight, total fat mass (FM) and (intra)-abdominal FM in normalweight and overweight/obese women before and after menopause. A structured electronic search was performed to find all publications relevant to our review. Stratified analyses were made of hormonal status (pre- vs. postmenopausal state), weight, HIIT modalities (cycling vs. running), programme duration (< or ≥8 weeks) and the methods used to measure body composition (dual-energy X-ray absorptiometry vs. computed tomography, Magnetic Resonance Imaging and others). A total of 38 studies involving 959 subjects were included. Our meta-analysis showed that overall HIIT programmes significantly decrease weight, total and abdominal FM in women. Both normal weight and overweight/obese women lost total FM after HIIT protocols whereas HIIT was only effective in decreasing abdominal FM in women with excess adiposity. When pre- and postmenopausal women were considered separately, the effect of HIIT on weight, total and abdominal FM were only significant before menopause. Cycling HIIT seemed more effective than running, especially in postmenopausal women, and training interventions longer than 8 weeks comprising three sessions were more efficient. HIIT is a successful strategy to lose weight and FM in normal weight and overweight/obese women. However, further studies are still needed to draw meaningful conclusions about the real effectiveness of HIIT protocols in postmenopausal women.

KEYWORDS

abdominal fat mass, fat mass, high-intensity interval training, menopause, women

1 | INTRODUCTION

Numerous studies in women show an increased risk of obesity in midlife (Kapoor, Collazo-Clavell, & Faubion, 2017; Lizcano & Guzmán, 2014). At menopause, this risk increases and with it obesity-related disorders such as hypertension, hypercholesterolaemia and type 2 diabetes (Monteleone, Mascagni, Giannini, Genazzani, & Simoncini,

2018). The greater occurrence of metabolic diseases is related to body composition changes, such as an increased amount of total body fat, a loss of lean body mass and changes in body fat mass (FM) distribution (Abildgaard et al., 2013). In premenopausal women, the total amount of abdominal fat (including visceral deposits) is lower and reduces the risk of metabolic syndrome (Palmer & Clegg, 2015). However, after menopause, body fat distribution shifts to a male profile ('android type',

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also called central obesity) thereby increasing the risk of developing metabolic syndrome and several chronic diseases such as cardio-vascular diseases (CVD) (Kapoor et al., 2017; Karvonen-Gutierrez & Kim, 2016).

The management of weight and FM loss in premenopausal and postmenopausal women is a major public health concern (Dolin & Kominiarek, 2018). To promote body weight and FM reductions, an energy imbalance is required (i.e. energy input < energy output). Data from the literature show that short- and long-term programmes based on nutritional recommendations alone are less effective than those that also include regular physical activity (Hassan, Latif, & Yacoub, 2012; Verheggen et al., 2016). Current international guidelines mostly recommend moderate-intensity continuous training (MICT) as the best strategy in both men and women to reduce body weight and FM, mainly because of the duration and intensity of the sessions, which promote fat mobilization and oxidation (Donnelly et al., 2009). However, without contesting its positive cardiovascular and metabolic effects, this training mode generally leads to little or no fat loss (Boutcher, 2011). High intensity interval raining (HIIT) - repeated bouts of high-intensity effort ranging between ~80% and 100% peak heart rate followed by less intense recovery times (MacInnis & Gibala, 2017; Weston, Wisløff, & Coombes, 2014) - has recently been proposed as a timeefficient modality to increase post-exercise lipid oxidation, which helps to reduce body weight and adipose tissue (Boutcher, 2011; Karstoft, Wallis, Pedersen, & Solomon, 2016; Wewege, van den Berg, Ward, & Keech, 2017) including (intra)-abdominal FM, the most dangerous one (Maillard et al., 2018).

Numerous publications and reviews are now available concerning HIIT-induced health benefits including body weight and FM losses (Maillard et al., 2018; Roy et al., 2018; Türk et al., 2017) but none of them focus specifically on women and none of them take into account the hormonal status of these women. Yet, it is well known that the oestrogen deficiency observed at menopause can alter metabolic adaptations to exercise (Isacco & Boisseau, 2017). For these reasons, the beneficial effects on body composition induced by HIIT could be altered in postmenopausal women and hence lead to inappropriate recommendations.

The aim of this review was to establish from these literature data the efficacy of HIIT in reducing weight, total and (intra)-abdominal FM in women before and after menopause. The analysis was applied to normal-weight and overweight/obese women. To arrive at practical recommendations, particular attention was paid to the type of the HIIT (cycling vs. running), the duration of the programmes (< or \ge 8 weeks) and the methods used to measure body composition.

2 | METHODS

2.1 | Literature search strategy

PubMed/MEDLINE, EMBASE, Google Scholar, Cochrane and Web of Science up to December 2019 were used to identify related studies. The keywords chosen were 'high intensity interval training' (HIIT),

New Findings

• What is the topic of this review?

A meta-analysis of the efficacy of high intensity interval training (HIIT) in reducing weight, total fat mass (FM) and (intra)-abdominal FM in normal-weight and overweight/obese women before and after menopause.

· What advances does it highlight?

HIIT programmes in women significantly decrease body weight and total and abdominal FM. Their effects are more evident in pre- than in postmenopausal women. Cycling HIIT seems more effective than running, especially in postmenopausal women, and training interventions longer than 8 weeks comprising three sessions a week should be promoted.

'high-intensity intermittent exercise' (HIIE), 'aerobic interval exercise' (AIE) or 'aerobic interval training' (AIT), with and without 'women', 'girl', 'female' or 'body composition', 'body fat', 'fat mass'. A manual search of reference lists and citations was conducted to find potentially relevant studies. Publications in English and French were retained for analysis.

2.2 | Inclusion and non-inclusion criteria

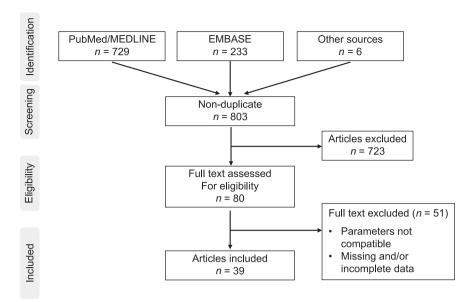
Inclusion criteria were (a) studies using HIIT protocols with running, cycling and elliptical modalities without restrictions to the duration of the protocol and the HIIT modality, and (b) adult women (≥18 years old) with normal weight, overweight or obesity. Only adult women were analysed because training adaptations and substrate use differ with sex and age. Non-inclusion criteria were (a) studies including energy restrictions or specific diets, (b) studies using supramaximal interval training (SIT) protocols, and (c) studies including high-level athletes. However, when publications referred incorrectly to 'SIT' or 'Wingate' protocols' (i.e. when subjects performed with an intensity level below 100% of the peak heart rate), the data were included. Pre-/postmenopausal women were categorized as such when the distinction was clearly mentioned in the publications.

2.3 Data extraction

For each study, the first author and second author (M.D. and F.M.) separately extracted the data from all the publications with advice from N.B. on selection criteria. We summarized the following information.

 Protocol characteristics: exercise type (running vs. cycling), HIIT protocol, duration and frequency.

FIGURE 1 Flow diagram of the systematic reviewing process



- Population characteristics: number of subjects, age, body mass index, body composition measurements, other information.
- Outcome measures: weight, total FM, abdominal FM and visceral FM.

Authors of the studies selected were contacted for missing values.

2.4 | Study quality

Study quality was assessed by M.D. and F.M. using a checklist adapted from Downs & Black (1998) and Keating, Johnson, Mielke, & Coombes (2017). All the studies were retained irrespective of the score obtained. Items included 19 questions about hypotheses, outcomes, patient characteristics (on the basis of clearly stated inclusion and exclusion criteria), randomization, interventions, lifestyle (habitual energy expenditure or diet), descriptions of patients lost to follow-up (studies with >10% dropout without characteristics reported scored 0), details of HIIT protocol (type and training duration), main findings, statistical method, assessment method accuracy and adverse events. If the answer to an item was 'yes', the item score was 1; if it was 'no' or 'unable to determine' the score was zero. If an item was unable to be determined, it was scored as zero. The highest possible score for quality was 19. The quality score of the studies was based on tertiles, for which 0-6 points is considered low quality, 6-12 points medium quality and 13-19 points high quality.

2.5 | Statistical analysis

After extraction, the data were compiled into software designed specifically for meta-analyses (Comprehensive Meta-Analysis, version 2; Biostat, Englewood, NJ, USA). Data included were sample size, and pre- and post-intervention values. The standardized mean differences

(paired SMD) were calculated to determine Cohen's d for each study, and Hedges' g was used to account for potential bias in small sample sizes. Effect sizes were calculated with a random-effects model (DerSimonian and Laird approach) that accounts for true variation in effects occurring from study to study and for random errors within a single study. The random-effects model was preferred to a fixed-effect model because certain experimental parameters had wide variation. The effect sizes were interpreted according to Cohen: <0.2, trivial; 0.2-0.3, small; 0.5, moderate; and >0.8, large (Cohen, 1992). A negative effect size value indicated that exercise decreased outcomes while a positive effect size value indicated that exercise increased outcomes. The Iš index was used to measure heterogeneity with 25%, 50% and 75% indicating low, moderate and high heterogeneity, respectively. To test sensitivity and whether results were biased by a particular study the analyses were conducted by excluding one study at a time. Funnel plots were used to assess publication bias. In the absence of bias, studies would normally be distributed evenly around the mean effect size because of random sampling error.

3 | RESULTS

3.1 Study selection

The search strategy identified 803 non-duplicate articles from electronic databases; 723 publications were excluded after title and/or abstract analysis. Of the remaining articles, 80 fulfilled our inclusion criteria. The total number of publications finally included in the meta-analysis was 38: 37 for total mass, 28 for total FM, 11 for abdominal FM and 8 for visceral FM (Figure 1). The mean quality score obtained after evaluation of each trial included in our meta-analysis was 14.5 \pm 2.3. Most of the studies were of high quality, having a score above 15 points.

Subject characteristics are summarized in Table 1. Overall, 959 subjects were included in the meta-analysis. In accordance with the

 TABLE 1
 Characteristics of studies and subjects included in the review

Study	Number of subjects in HIIT group	Age (years)	BMI (kg m ⁻²)	Body composition measurements	Other	Hormonal status
Almenning et al., 2015	10	25.5 ± 5.0	26.1 ± 6.5	Impedance	Polycystic ovary syndrome	Premenopausal
Alvarez et al., 2016	14	46.0±3.0	30.8 ± 1.0	Circumferences and skinfolds	Sedentary overweight/obese women, T2D	Premenopausal
Álvarez et al., 2017	49	40.0 ± 6.0	29.5 ± 3.7	Circumferences and skinfolds	Overweight/obese women, high IR but no T2D	Premenopausal
Álvarez et al., 2017	29	35.0 ± 9.0	27.8 ± 2.8	Circumferences and skinfolds	Overweight/obese women, low IR but no T2D	Premenopausal
Alvarez et al., 2018	20	45.6 ± 11.0	30.6 ± 4.0	Circumferences and skinfolds	Overweight/obese women, T2D	UK
Alvarez et al., 2018	19	42.2 ± 9.0	28.5 ± 2.0	Circumferences and skinfolds	Overweight/obese women, dyslipidaemic	UK
Alvarez et al., 2018	25	42.3±10.0	29.0 ± 3.0	Circumferences and skinfolds	Overweight/obese women, dyslipidemic and high glucose	N.
Alvarez et al., 2018	32	41.4 ± 9.0	28.2 ± 4.0	Circumferences and skinfolds	Overweight/obese healthy women	N
Alvarez et al., 2018	63	35.2 ± 5.1	31.9 ± 5.8	Impedance and skinfolds	Sedentary women prehypertensive	Premenopausal
Alvarez et al., 2018	79	36.6 ± 5.8	30.0 ± 6.6	Impedance and skinfolds	Sedentary women normotensive	Premenopausal
Alves et al., 2017 (1)	10	25.5 ± 5.0	25.4 ± 3.9	Skinfolds	Untrained women	Premenopausal
Alves et al., 2017 (2)	10	25.5 ± 5.0	26.0 ± 4.4	Skinfolds	Untrained women	Premenopausal
Arad et al., 2015	14	29.0 ± 4.0	32.5 ± 3.6	DXA VAT: MRI	Healthy overweight/obese women	Premenopausal
Astorino et al., 2013	11	22.7 ± 5.4	25.0 ± 4.3	Circumferences and skinfolds	Healthy sedentary women	Premenopausal
Bhati et al., 2017 (1)	18	22.0 ± 0.5	20.3 ± 0.4	Impedance	Sedentary young women	Premenopausal
Bhati et al., 2017 (2)	17	22.1 ± 0.4	22.4 ± 0.5	Impedance	Sedentary young women	Premenopausal
Bouri et al., 2015	7	23.0 ± 2.5	29.2 ± 2.1	Impedance	Overweight sedentary women	Premenopausal
Boutcher et al., 2019	20	54.1 ± 3.6	28.3 ± 3.7	DXA	Overweight/obese postmenopausal women	Postmenopausal
Chrøis et al., 2019	11	63.0 ± 1.0	31.0 ± 1.0	DXA	Overweight/obese postmenopausal women	Postmenopausal
Clark et al., 2019	17	37.5 ± 10.5	ž	Plesthymosgraphy	Obese women	UK
Connolly et al., 2017	16chr	44.0 ± 7.0	25.3 ± 3.9	Plesthymosgraphy	Inactive premenopausal women	Premenopausal
Coquart et al., 2008 (a)	10	52.0 ± 7.3	38.2 ± 7.9	Impedance	Obese women T2D	λ
Coquart et al., 2008 (b)	10	51.2 ± 6.5	37.0 ± 3.8	Impedance	Obese women without T2D	UK
Dupuit et al., 2020	10	59.9 ± 5.9	31.5 ± 4.3	DXA	Overweight/obese postmenopausal women	Postmenopausal
Eimarieskandari et al., 2012	7	22.3 ± 0.9	29.2 ± 0.8	Impedance	Obese young girl	Premenopausal
Gillen et al., 2013 (a)	8	27.0 ± 7.0	29.0 ± 3.0	DXA	Overweight/obese women, fed	Premenopausal
Gillen et al., 2013 (b)	8	27.0 ± 9.0	29.0 ± 4.0	DXA	Overweight/obese women, fasted	Premenopausal
Henke et al., 2018	10	58.2 ± 3.1	31.7 ± 1.3	Skinfolds	Sedentary obese women	Postmenopausal (Continues)

TABLE 1 (Continued)

Study	Number of subjects in HIIT group	Age (years)	BMI (kg m ⁻²)	Body composition measurements	Other	Hormonal status
Hornbuckle et al., 2017	16	32.1 ± 7.0	36.8±4.3	DXA	Overweight and obese African-American women	Premenopausal
Hutchison et al., 2011 (a)	20	29.5 ± 1.4	37.4 ± 1.5	DXA VAT: CT scan	Overweight women, polycystic ovary syndrome	Premenopausal
Hutchison et al., 2011 (b)	14	35.0 ± 4.1	35.7 ± 4.9	DXA VAT: CT scan	Overweight women, no polycystic ovary syndrome	Premenopausal
Kong et al., 2016	11	19.8 ± 0.8	25.5 ± 2.1	DXA	Overweight and obese young women	Premenopausal
Maillard et al., 2016	ω	69.0±2.8	32.6 ± 4.8	DXA VAT: CT scan	T2D postmenopausal	Postmenopausal
Mandrup et al., 2017 (a)	39	49.2 ± 0.6	23.9 ± 0.7	DXA	Healthy non-obese women	Premenopausal
Mandrup et al., 2017 (b)	38	53.4 ± 1.0	23.7 ± 0.8	DXA	Healthy overweight women	Postmenopausal
Mirghani & Yousefi, (1)	œ	33.0 ± 5.3	32.2 ± 3.8	Circumferences and skinfolds	Overweight/obese women	Premenopausal
Mirghani & Yousefi, 2014 (2)	80	32.0 ± 5.4	28.1 ± 3.3	Circumferences and skinfolds	Overweight/obese women	Premenopausal
Naves et al., 2018	34	31.0 ± 6.0	24.5 ± 3.3	Circumferences and skinfolds	Healthy women	Premenopausal
Nie et al., 2019	12	20.0 ± 1.0	27.0 ± 2.8	Impedance	Sedentary women	Premenopausal
Panissa et al., 2016	11	28.4 ± 12.5	25.94 ± 4.1	Skinfolds	Untrained women	Premenopausal
Sandstad et al., 2015	7	32.4 ± 8.3	24.8 ± 4.9	Impedance	Rheumatic disease	Premenopausal
Sijie et al., 2012	20	19.8 ± 1.0	27.7 ± 1.9	DXA	Overweight/obese women students	Premenopausal
Smith-Ryan et al., 2016 (1)	11	32.2 ± 12.8	33.9 ± 6.1	DXA	Overweight/obese women	Premenopausal
Smith-Ryan et al., 2016 (2)	11	33.6 ± 11.6	28.6 ± 3.7	DXA	Overweight/obese women	Premenopausal
Steckling et al., 2016	17	54.2 ± 6.2	30.7 ± 5.0	Impedance	Untrained metabolic syndrome women	Premenopausal
Steckling et al., 2019	17	53.9 ± 5.9	31.3 ± 4.9	DXA	Metabolic syndrome women	Postmenopausal
Sun et al., 2018	16	21.5 ± 1.8	26.0 ± 2.4	Impedance	Overweight/obese women	Premenopausal
Tong et al., 2018	18	21.3 ± 1.0	ΛΥ	DXA	University student women	Premenopausal
Trapp et al., 2008	15	22.4 ± 2.7	24.4 ± 5.8	DXA	Healthy normal-weight women	Premenopausal
Zapata-Lamana et al.,	16	23.3 ± 3.8	33.8 ± 3.8	DXA	Young overweight/obese women	Premenopausal
Zhang et al., 2015	14	21.0 ± 1.0	25.8 ± 2.7	Impedance VAT: CT scan	Overweightwomen	Premenopausal
Zhang et al., 2017	16	21.5 ± 1.7	25.4 ± 2.4	DXA VAT: CT scan	Overweightwomen	Premenopausal

protocols. (a)-(d) represent the same study but different population characteristics. BMI, body mass index; CT, computed tomography; DXA, dual-energy X-ray absorptiometry; FM, fat mass; HIIT, high-intensity interval training; IR, insulin resistance; MRI, magnetic resonance imaging; T2D, type 2 diabetes; VAT, visceral adipose tissue. UK: unknown. Data expressed as means ± standard deviation (SD); in instances where the results were present as mean ± standard error (SEM), SEM was converted to SD. (1) and (2) represent the same study but different HIIT

inclusion criteria, women were adults, with a mean age ranging from 19.8 ± 0.8 to 69.0 ± 2.8 years. All studies except five had recruited overweight or obese women, whose body mass index (BMI) ranged from 20.3 ± 0.4 to 38.2 ± 7.9 kg m⁻š. Some women had diseases that could have influenced the effects of physical activity: type 2 diabetes (n = 4), dyslipidaemia (n = 1), polycystic ovary syndrome (n = 2), hormonal state (menopause) (n = 6), metabolic syndrome (n = 2) and rheumatic disease (n = 1).

3.2 | Hormonal status of the women

Most studies (n=33) were performed in premenopausal women but generally without any details concerning the period of the menstrual cycle or the use of contraceptive pills. Two studies did not give any information about the hormonal status of the women (i.e. pre- or postmenopausal) (Table 1). Thus, only seven studies in our analysis included postmenopausal women.

3.3 | Body composition assessments

Fifteen studies used dual-energy X-ray absorptiometry to determine whole body FM (Table 1). Other studies used less accurate and/or repeatable methods such as impedance (n=11), plethysmography (n=2) or circumference and skinfold measurements (n=9), the last of which was recognized as the least reliable. Computed tomography (CT) and magnetic resonance imaging (MRI) were widely used to assess abdominal or visceral FM. The most recent DXA scans can also measure abdominal FM in different anatomical regions and estimate visceral FM content (Sawyer et al., 2016).

3.4 | HIIT programme characteristics

The HIIT programmes are described in Table 2. Twenty-six HIIT studies used cycling and 12 running, with one offering a choice between the two. Only one study tested an elliptical type. The most widely used protocol consisted of alternate bouts of 4 min at high intensity followed by 3 min of recovery (n=13). Other programmes used shorter times (8 s or 1 min) at high intensity. When specified, recovery was generally active in all studies. The programmes ranged in duration from 3 to 16 weeks but usually lasted at least 10 weeks. In terms of frequency, most studies used three sessions a week (n=32), lasting <8 weeks (n=6) or \geq 8 weeks (n=33).

3.5 | Meta-analysis

3.5.1 | Influence of HIIT on body weight, total FM and (intra)-abdominal FM and impact of hormonal status (before and after menopause)

Overall, HIIT interventions decreased weight (effect size (ES): -0.19 (95% CI: -0.30 to -0.09), $P < 10^{-3}$), total FM (ES: -0.30 (95% CI:

-0.43 to -0.17), $P < 10^{-3}$), abdominal FM (ES: -0.22 (95% CI: -0.42 to -0.02), P < 0.03) but only tended to reduce visceral adipose tissue (ES: -0.25 (95% CI: -0.53 to 0.03), P = 0.078) (Figure 2).

Stratified analysis of hormonal status showed a decrease in body weight after HIIT in premenopausal women (ES: -0.22 (95% CI: -0.35 to -0.10), P < 0.001) but not in postmenopausal women (ES: -0.09 (95% CI: -0.35 to 0.18), P = 0.51) (Figure 2a). Total FM was significantly reduced in premenopausal women (ES: -0.23 (95% CI: -0.48 to -0.03), P < 0.001) whereas only a trend was observed in postmenopausal women (P = 0.079) (Figure 2b). HIIT-induced abdominal FM loss was observed only in premenopausal women (P = 0.021) (Figure 2c). HIIT tended to reduce visceral abdominal adiposity in premenopausal women (P = 0.06), but no effect was observed in postmenopausal women (Figure 2d).

3.5.2 | Influence of BMI on HIIT-induced body composition changes

Both normal weight (ES: -0.61 (95% CI: -0.87 to -0.35), P < 0.001) and overweight/obese (ES: -0.20 (95% CI: -0.35 to -0.05), P = 0.01) women lost total FM with HIIT programmes (data not shown). Overall, HIIT impact on total FM differed according to BMI with a greater effect on overweight/obese than on normal weight women (P = 0.011). This subanalysis had in fact to be limited to premenopausal women (analysis in this subgroup: ES: -0.25 (95% CI: -0.43 to -0.06), P = 0.009) since all the studies performed with postmenopausal women included overweight or obese subjects.

HIIT was only effective in decreasing abdominal FM in women with an excess of weight at baseline (ES: -0.22 (95% CI: -0.43 to -0.01), P = 0.043). For the same reason as above, this result is restricted to premenopausal women (ES: -0.29 (95% CI: -0.56 to -0.02), P = 0.033).

3.5.3 | Effect of HIIT type on body composition changes

Although both modalities were effective (P < 0.001), stratified analysis comparing exercise type showed that cycling (P = 0.007) was more effective than running (P = 0.01) in reducing body weight (data not shown). Concerning total FM, the analysis showed that only cycling was effective in reducing total FM (ES: -0.34 (95% CI: -0.49 to -0.19), P < 0.001) and that for running simply a trend was observed (ES: -0.21 (95% CI: -0.46 to 0.04), P = 0.09) (Figure 3). When pre- and postmenopausal women were separated into two distinct groups and subdivided according to exercise mode, the same conclusions emerged in each group. However, the trend for running was more pronounced in premenopausal women (P = 0.053) and was not observed in postmenopausal women (P = 0.957).

Only cycling reduced abdominal FM (ES: -0.27 (95% CI: -0.50 to -0.04), P = 0.02) whereas no effect was found in running protocols (ES: -0.08 (95% CI: -0.49 to 0.33), P = 0.69) (data not shown). No HIIT effect was observed with cycling (ES: -0.20 (95% CI: -0.54 to 0.14),

TABLE 2 HIIT protocol details

Study	Exercise type	HIIT protocol	Duration	Frequency (week ⁻¹)
Almenning et al., 2015	Running or cycling	$4 \times [4 \text{ min } (90-95\% HR_{max})/3 min (70\% HR_{max})] + 1/week: 10 min: 1 min (100\% of max effort)/1 min$	10 weeks	3
Álvarez et al., 2017	Running	$8 \times [60 \text{ s } (90-100\% \text{ HR}_{max})/120 \text{ s } (70\% \text{ HR}_{max}) \text{ recovery}]$	16 weeks	3
Álvarez et al., 2017	Cycling	8- $12 \times [60 \text{ s} (70-100\% \text{ HR}_{max})/120 \text{ s passive}]$	10 weeks	3
Alvarez et al., 2018	Running	$8 \times [60 \text{ s } (90-100\% \text{ HR}_{max})/120 \text{ s } (70\% \text{ HR}_{max}) \text{ R}]$	16 weeks	3
Alvarez et al., 2018	Cycling	7–10 min [60 s (8–10 CR10 Bjorg)–120 s passive R]	16 weeks	3
Alves et al., 2017 (1)	Cycling	$15 \times [1 \text{ min } (90\% \text{ HR}_{max})/30 \text{ s } (60\% \text{ HR}_{max})]$	6 weeks	3
Alves et al., 2017 (2)	Cycling	$45 \times [20 \text{ s} (90\% \text{ HR}_{max})/10 \text{ s} (60\% \text{ HR}_{max})]$	6 weeks	3
Arad et al., 2015	Cycling	24 min [30-60 s (75-90% HRR)/180-210 (50% HRR)]	14 weeks	3
Astorino et al., 2013	Cycling	$10 \times [60 \text{ s} (80-90\% \text{ HR}_{max})/75 \text{ s} (40\% \text{ HR}_{max})]$	12 weeks	3
Bhati et al., 2017 (1)	Running	LVHIIT: $1 \times [4 \text{ min } (85-95\% \text{ HR}_{max})/3 \text{ min } (70\% \text{ HR}_{max})]$	6 weeks	3
Bhati et al., 2017 (2)	Running	HVHIIT: $4 \times [4 \text{ min} (85-95\% \text{ HR}_{max})/3 \text{ min} (70\% \text{ HR}_{max})]$	6 weeks	3
Bouri et al., 2015	Running	$4 \times [4 \text{ min} (85-95\% \text{ HR}_{max})/3 \text{ min} (50-70\% \text{ HR}_{max})]$	8 weeks	3
Boutcher et al., 2019	Cycling	20 min: $60 \times [8 \text{ s} (80-85\% \text{ HR}_{max}/12 \text{ s active R})]$	8 weeks	3
Chrøis et al., 2019	Cycling	$5-9 \times [1 \text{ min } (85\% \text{ HR}_{max})/1 \text{ min } 30 \text{ active or passive }$ recovery]	6 weeks	3
Clark et al., 2019 (1)	Cycling	$10 \times [60 \text{ s}/60 \text{ s} (70-85\% \text{ of PPO})]$	6 weeks	3
Clark et al., 2019 (2)	Cycling	$6-10 \times [60 \text{ s}-120 \text{ s}/60 \text{ s}-120 \text{ s} (65-110\% \text{ of PPO})]$	6 weeks	3
Connolly et al., 2017	Cycling	$5 \times 5 \min [10 \text{ s} (>90\% \text{ HR}_{max})/20 \text{ s} (50-60\% \text{ HR}_{max})/30 \text{ s}$	12 weeks	3
Coquart et al., 2008	Cycling	32 min: 2 min (80% VT)/2 min (120% VT)	10 weeks	3
Dupuit et al., 2020	Cycling	20 min: $60 \times [8 \text{ s} (80-90\% \text{ HR}_{max}/12 \text{ s active R})]$	12 weeks	3
Eimarieskandari et al., 2012	Running	25 min: [4 min (80–90% \dot{V}_{O_2peak} (85–95% of HR_{peak}))/ 3 min (50–60% of \dot{V}_{O_2peak} (50–70% of HR_{peak})) R]	8 weeks	3
Gillen et al., 2013	Cycling	$10 \times [60 \text{ s } (90\% \text{ HR}_{max})/60 \text{ s}]$	6 weeks	3
Henke et al., 2018	Cycling	$10 \times [60 \text{ s } (85-90\% \text{ HR}_{max})/75 \text{ s } (40\% \text{ HR}_{max}) \text{ R}]$	4 weeks	2
Hornbuckle et al., 2017	Running	32 min: 3 min (60-70% of HR _{max})/1 min (80-90%) HR _{max}	16 weeks	3
Hutchison et al., 2011	Running	$6-8 \times [5 \text{ min } (95-100\% \text{ HR}_{\text{max}})/2-1 \text{ min R}) + 1 \text{ session: } 60 \text{ min at } 75-85\% \text{ HR}_{\text{max}} (70\% \dot{V}_{\text{O}_2\text{max}})$	12 weeks	3
Kong et al., 2016	Cycling	60 × [8 s/12 s) R].	5 weeks	4
Maillard et al., 2016	Cycling	20 min: $60 \times [8 \text{ s} (77-83\% \text{ HR}_{\text{max}}/12 \text{ s active R})]$	16 weeks	2
Mandrup et al., 2017	Cycling	$15 \times [2 \text{ min}(82-95\% \text{ of HR}_{max})]/1 \text{ min R}]$	12 weeks	3
Mirghani & Yousefi, (1)	Running	10 × [60 s (80% HRR)/60 s active R]	4 weeks	3
Mirghani & Yousefi, 2014 (2)	Running	10 × [60 s (80% HRR)/30 s active R]	4 weeks	3
Naves et al., 2018	Cycling	$4 \times [4 \text{ min} (85-95\% \text{ HR}_{max})/3 \text{ min} (60\% \text{ HR}_{max})]$	12 weeks	2
Nie et al., 2019	Cycling	$4 \times [4 \text{ min } (90\% \text{ HR}_{\text{max}})/3 \text{ min passive R}]$	8 weeks	3
Panissa et al., 2016	Cycling	$15 \times [1 \text{ min}(90\% \text{ of HR}_{max})/30 \text{ s } (60\% \text{ HR}_{max}) \text{ R}]$	6 weeks	3
Sandstad et al., 2015	Cycling	$4 \times [4 \text{ min } (85-95\% \text{ HR}_{max})/3 \text{ min } (70\% \text{ HR}_{max})]$	10 weeks	2
Sijie et al., 2012	Running	$5 \times [3 \text{ min } (85\% \dot{V}_{O_2 \text{max}})/3 \text{ min } (50\% \dot{V}_{O_2 \text{max}})]$	12 weeks	5
Smith-Ryan et al., 2016 (1)	Cycling	$10 \times [60 \text{ s } (90\% \dot{V}_{O_2 \text{peak}})/60 \text{ s active R}]$	3 weeks	3
Smith-Ryan et al., 2016 (2)	Cycling	$5 \times [2 \text{ min } (80-100\% \dot{V}_{O_2 \text{peak}})/60 \text{ s active R}]$	3 weeks	3
Steckling et al., 2016	Running	4 × [4 min (90% HR _{max})/3 min (70% HR _{max}) R]	12 weeks	3
Steckling et al., 2019	Running	4×[4 min (90% HR _{max})/3 min (70% HR _{max}) R]	12 weeks	3
Sun et al., 2018	Cycling	$9 \times [4 \text{ min } (90\% \dot{V}_{O_2 \text{max}})/3 \text{ min } (60\% \text{ HR}_{\text{max}}) \text{ R}]$	12 weeks	3
Tong et al., 2018	Cycling	400 kJ: 4 min (90% V _{O₂max})/3 min R)	12 weeks	3

(Continues)

TABLE 2 (Continued)

Study	Exercise type	HIIT protocol	Duration	Frequency (week ⁻¹)
Trapp et al., 2008	Cycling	20 min: 60 × (8 s/12 s)	15 weeks	3
Zapata-Lamana et al.,	Cycling	$4 \times [4 \times [1 \text{ min } (90\% \dot{V}_{O_2 \text{peak}})/2 \text{ min } R] 4 \text{ min } R]$	12 weeks	3
Zhang et al., 2015	Running	$4\times[4\text{min}(85-95\%)\text{HR}_{\text{peak}})/3\text{min}(50-60\%\text{HR}_{\text{peak}})$ with 7 min rest between sets	12 weeks	4
Zhang et al., 2017	Cycling	300 kJ: 4 min (90% $\dot{V}_{O_2 max}$)/3 min R)	12 weeks	3

(1) and (2) represent the same study but different HIIT protocols. HIIT, high-intensity interval training; HR, heart rate; HRR, heart rate reserve; HV HIIT, High volume high-intensity interval training; PPO, peak power output; R, recovery; \dot{V}_{O_2} , oxygen consumption; VT, ventilatory threshold.

P=0.24) or with running programmes (ES: -0.35 (95% CI: -0.84 to 0.14), P=0.16) on the reduction of visceral FM. The same conclusions emerged for abdominal and visceral FM loss in premenopausal women (data not shown) and were not applicable to postmenopausal women.

3.5.4 ☐ Effect of HIIT programme duration on body composition changes

Only studies using three sessions a week were retained in this analysis. Comparison of the duration of HIIT programmes showed that only training interventions longer than 8 weeks had significant effects on body weight (ES: -0.21 (95% CI: -0.34 to -0.09), P < 0.001) (Figure 4a). While interventions shorter than 8 weeks are effective in reducing total FM (ES: -0.27 (95% CI: -0.52 to -0.03), P = 0.03), the effects are greater when training comprises or exceeds 8 weeks (ES: -0.33 (95% CI: -0.49 to -0.17), P < 0.001) (data not shown). Only interventions \geq 8 weeks significantly reduced abdominal FM (ES: -0.28 (95% CI: -0.51 to -0.04), P = 0.02) (Figure 4b). Training duration had no effect on visceral FM.

As only one study performed with postmenopausal women lasted less than 8 weeks, the significance of these results is predominantly applicable to premenopausal women.

3.5.5 | Body composition assessments

Total FM loss was significant when DXA (P < 0.001) or bioelectrical impedance analysis (P = 0.01) was used as the measurement method but not with plethysmography or the skinfold test (Figure 5a). Only CT (ES: -0.47 (95% CI: -0.84 to -0.09), P = 0.015) detected reductions in abdominal FM after HIIT programmes (Figure 5b).

4 | DISCUSSION

The present study is the first meta-analysis to assess the effect of HIIT on body weight, total FM and (intra)-abdominal FM losses in women and is also the first to compare these effects before and after menopause. The analysis involved 959 women aged between

 19.8 ± 0.8 and 69 ± 2.8 years, with a BMI between 20.3 ± 0.4 and 38.2 ± 7.9 kg m⁻². Our results showed that HIIT programmes, especially cycling interventions, of a duration of at least 8 weeks are effective in reducing total and (intra)-abdominal FM in normal weight and overweight/obese women. The effects of HIIT were more evident in pre- than in postmenopausal women.

Premenopausal women accumulate fat in a metabolically healthier gluteo-femoral pattern than men, which lowers their risk of metabolic syndrome development (Wajchenberg et al., 2000). After menopause, women experience a significant increase in total FM and a redistribution of adipose tissue, which leads to increased abdominal fat deposition (Rettberg et al., 2014). Importantly, the abdominal FM gain in postmenopausal women tends to be visceral and not subcutaneous (Lovejoy et al., 2008), which is detrimental since visceral FM is known to be the most dangerous with regard to CVD risks (Wajchenberg et al., 2000). If the mechanisms of the increase in total and visceral FM during menopause are still not clearly elucidated, one potential candidate is probably the rapid fall in oestrogen levels (Kozakowski et al., 2017). Firstly oestrogens activate catabolic and anorexigenic signals in the brain, similar to those recruited by the anti-obesity hormone leptin (Gao et al., 2007). Secondly, it has been demonstrated that oestrogens increase fat oxidation in skeletal muscle and inhibit hepatic and muscle lipogenesis (Foryst-Ludwig et al., 2010). Finally, oestradiol (the predominant oestrogen) when binding to oestrogen receptor α (ER α) inhibits adrenaline-stimulated lipolysis in human subcutaneous fat cells by increasing the number of α 2-adrenergic anti-lipolytic receptors (Pedersen et al., 2004). This could explain how oestrogens are related to typical female subcutaneous adipose tissue distribution before menopause because visceral fat deposits are not subject to the same inhibition. In addition, it was found that activation of the ER α receptors could stimulate adrenergic β receptors in the visceral tissue, enhancing the process of lipolysis and in this way reducing abdominal FM in premenopausal women (Kozakowski et al., 2017). These observations explain why oestrogen deficiency during menopause is associated with an increase in total FM and more specifically, in visceral FM (Babaei et al., 2017). Alongside these adaptations, postmenopausal women have a lower resting metabolic rate, a decrease in total energy expenditure, and reduced of fat oxidation during prolonged exercise (Gavin, Kohrt, Klemm, & Melanson, 2018; Poehlman et al., 2002). Here again, oestrogen deficiency probably plays a central role by

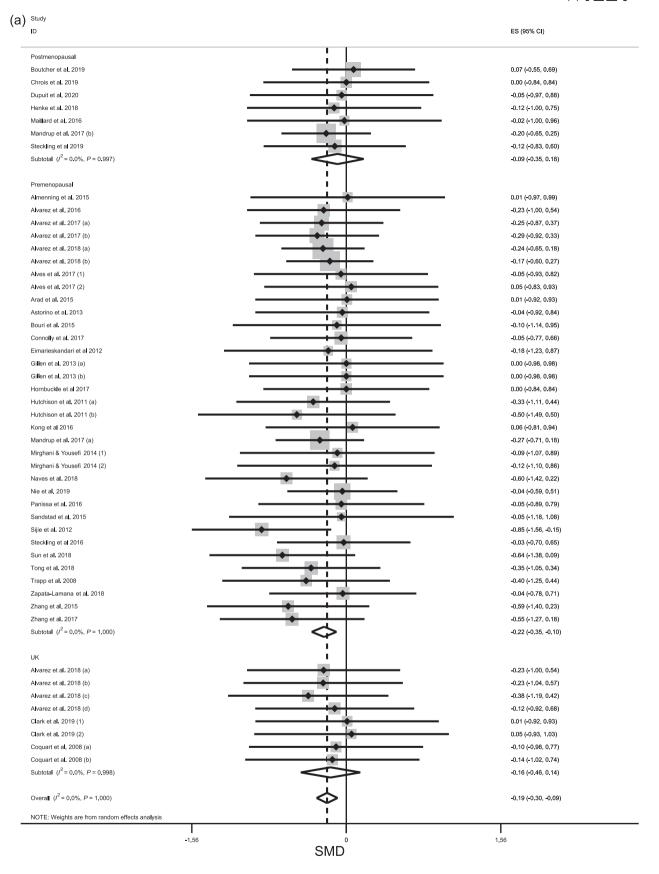


FIGURE 2 Forest plot for the effect of high-intensity interval training on body weight (kg) (a), total fat mass (b), abdominal fat mass (c) and visceral fat mass (d) with stratified analysis of hormonal status. (1) and (2) represent the same study but different HIIT protocols. (a)–(d) represent the same study but different analyses of abdominal fat mass. UK: unknown (hormonal status)



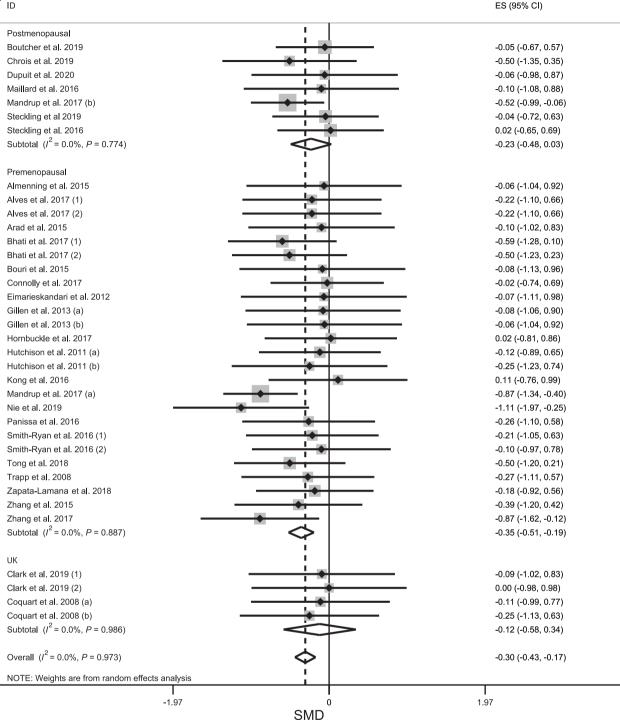


FIGURE 2 Continued

decreasing fat-free mass including muscle mass (Douchi et al., 2002), by decreasing energy expenditure and regulating brown fat activity (Gavin et al., 2018), and by promoting carbohydrate oxidation rates during prolonged exercise (Isacco & Boisseau 2017). Thus, overall, the lack of oestrogens associated with menopause predisposes women to an excess of body fat including visceral FM (Lovejoy et al., 2008) and could alter regular exercise-induced FM loss (Isacco & Boisseau 2017).

Although literature data show that the combination of diet and physical activity is the best way for promoting longer-term weight and/or FM loss (Verheggen et al., 2016), exercise alone can improve body composition if the energy expenditure leads finally to a negative energy balance (van Gemert et al., 2019; Verheggen et al., 2016). In the last position stand by the American College of Sports Medicine (ACSM) (Donnelly et al., 2009), moderate-intensity physical activity (between 150 and 250 min week⁻¹) is recommended for preventing weight gain.

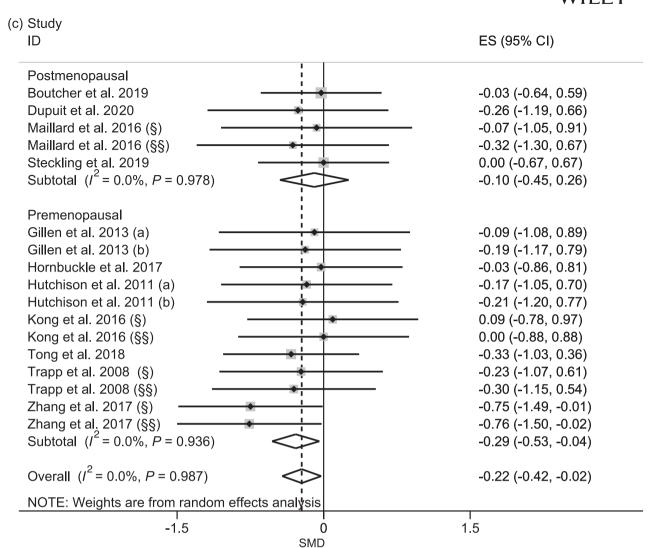
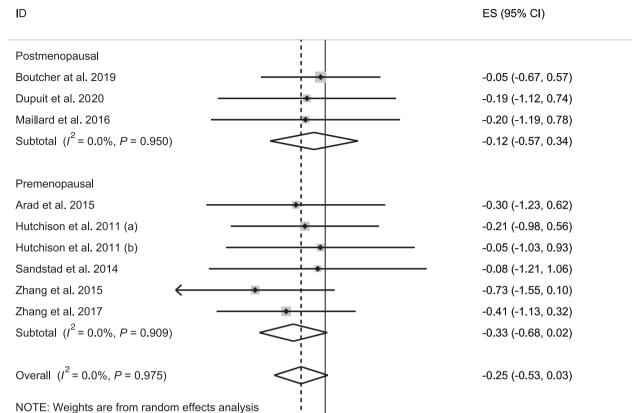


FIGURE 2 Continued

and more exercise for providing significant weight loss. Whatever the population involved, HIIT is now also recognized as a well-tolerated, safe and time-efficient strategy (Kessler et al., 2012; Taylor et al., 2017; Weston et al., 2014) to reduce body weight, total FM, and especially (intra)-abdominal adipose tissue (Maillard et al., 2018). As oestrogen deficiency promotes total and visceral FM gain, HIIT could be an interesting modality for women, particularly after menopause. All metaanalyses of the effects of HIIT on total and (intra)-abdominal FM loss were performed without distinction of sex except in the meta-analysis of Maillard et al., (2018) who stated that 'no sex-related effect was found for HIIT-induced reduction in total and abdominal or visceral FM'. However, the authors did not give any details on the hormonal status of the women, thereby overlooking the potential impact of oestrogen deficiency during menopause. Our review is thus the first to focus on the effects of HIIT on adipose tissue alterations in the female population and to consider separately these effects in pre- and postmenopausal women.

When all the women were pooled together, our meta-analysis confirmed that HIIT programmes significantly decrease body weight, total and abdominal FM and tend to reduce visceral FM in the female population. Both normal weight and overweight/obese women lost total FM after HIIT protocols whereas HIIT was only effective in decreasing abdominal FM in women with excess adiposity (BMI > 25). When pre- and postmenopausal women were separated from a stratified analysis, the effect of HIIT on weight and total FM was only significant before menopause (with a tendency after menopause for FM, P = 0.079). Likewise, HIIT-induced abdominal FM loss was observed only in premenopausal women. From these results, HIIT could be suspected of being less effective in reducing adipose tissue in postmenopausal women. The mechanisms underlying HIIT-induced significant total and (intra)-abdominal FM loss are not completely elucidated but could partly be the result of increased catecholamine production, leading to significant lipolysis during exercise, followed by higher post-exercise fat oxidation (Maillard et al., 2018). As overall Study



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FIGURE 2 Continued

oestrogen deficiency decreases the proportion of lipid metabolism in energy supply during exercise and limits fat oxidation under resting conditions (Gavin et al., 2018; Isacco & Boisseau 2017; Vieira-Potter et al., 2015), theoretically, postmenopausal women could lose less adipose tissue than premenopausal women after a few weeks of HIIT. However, the very small number of publications involving postmenopausal women makes interpretation difficult and further studies would be required for any definite conclusions to be drawn. A case in point is the report of Maillard et al., (2016), which showed that postmenopausal women performing a 16-week HIIT programme (60 × 8 s at 80-90% of peak heart rate, 12 s active recovery; 2 times a week) decreased their abdominal FM (DXA measurement from L1-L2 to the pubic rami). This finding emerged from a pre- vs. post-intervention comparison of two groups, one performing MICT and the other HIIT, using a two-way analysis of variance with repeated measures. When the HIIT group was considered separately, however, our stratified analysis failed to detect a HIIT-induced effect on abdominal FM loss in postmenopausal women. Another study performed by our laboratory also showed that a 12-week HIIT programme ($60 \times 8 \text{ s}$ at 80-90% of peak heart rate, 12 s active recovery) performed three times a week in 10 postmenopausal women resulted in a significant decrease in total ($-3.06\% \pm 4.2$) and abdominal ($-7.4\% \pm 2.8$) FM (Dupuit et al., 2020). Thus, a larger sample of postmenopausal women

-1.55

would have been probably necessary to reach a significant threshold in this meta-analysis and more data would be needed to establish whether oestrogen deficiency requires a greater total amount of interval training to achieve a comparable remodelling of body composition.

1.55

Overall, our meta-analysis evidenced a significant effect of cycling HIIT on FM loss but only a trend appeared with running. Similarly, cycling HIIT was effective in reducing abdominal FM whereas no effect was found with running HIIT. When pre- and postmenopausal women were considered separately and studies subdivided according to exercise mode, the same conclusions emerged in each group. However, whereas the trend for running almost reached a level of significance for total FM in premenopausal women (P = 0.053), no effect was observed in postmenopausal women (P = 0.957). Running elicits a greater cardiorespiratory response (\dot{V}_{O_2} and heart rate) than cycling during incremental and submaximal exercise, at matched relative and absolute workloads above and below the anaerobic threshold (Abrantes et al., 2012; Scott et al., 2006). However, when selecting running vs. cycling to perform a session of HIIT, workloads are inherently different (Kriel, Askew, & Solomon, 2018). One possible explanation is that, at a similar percentage of maximal heart rate or $\dot{V}_{\text{O}_{2}\text{max}}\text{,}$ there is a greater catecholamine release during cycling, which would lead to higher lipolysis via the activation of β -adrenergic

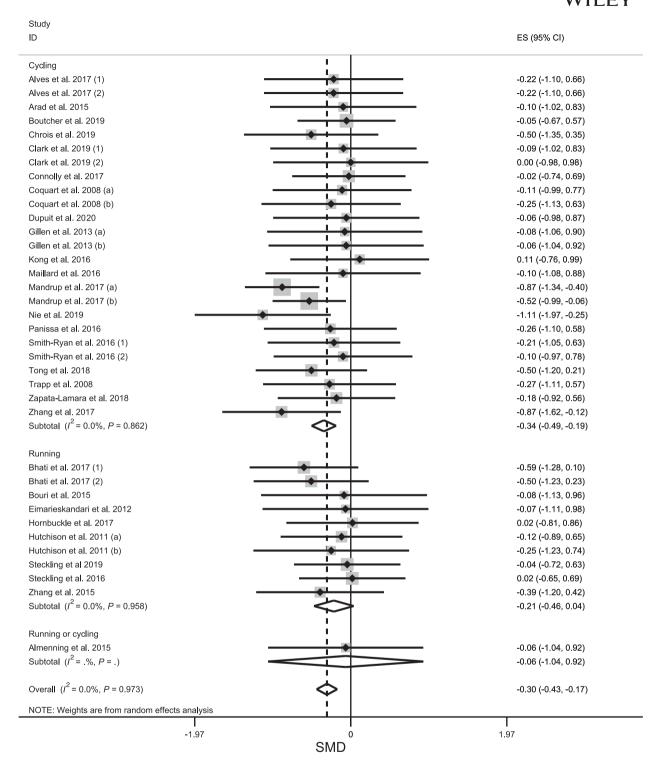


FIGURE 3 Forest plot for the effect of high-intensity interval training on total fat mass with stratified analysis of training type. (1) and (2) represent the same study but different HIIT protocols. (a) and (b) represent the same study but different population characteristics

receptors. However, Nieman et al., (1998) did not observe any greater catecholamine release in cycling vs. running protocols. In our meta-analysis, the heterogeneous nature of the HIIT programmes made the results difficult to interpret. In practical terms, however, it is possible that cycling is more suitable in sedentary women with overweight/obesity owing to the stress on joints caused by running and

the lack of compliance sometimes observed with running programmes after the age of 50 years (Prakash et al., 2017).

Overall comparison of HIIT programmes comprising three sessions a week showed that only training interventions longer than 8 weeks had significant effects on body weight. Nevertheless, interventions shorter than 8 weeks were effective in reducing total FM (but to a lesser

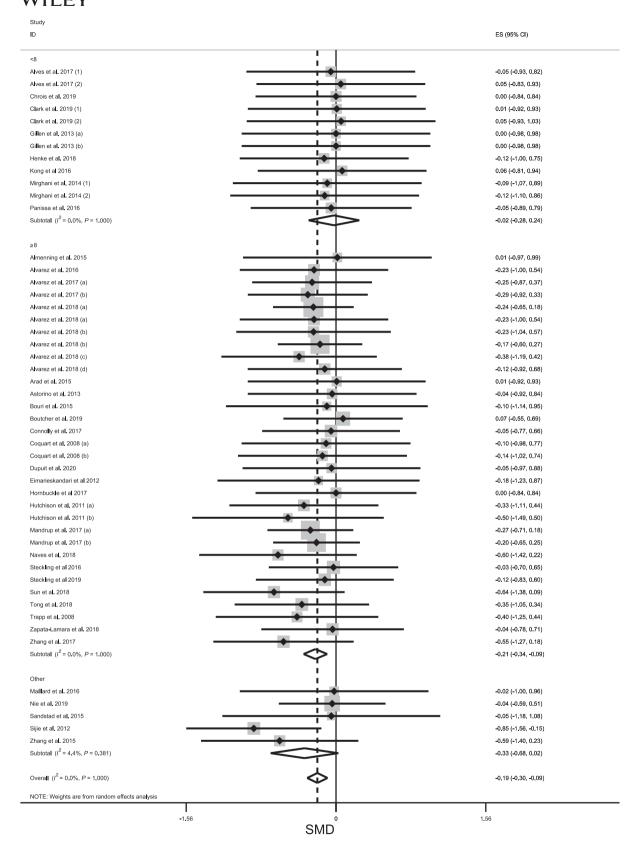


FIGURE 4 Forest plot for the effect of high-intensity interval training on body weight (kg) (a) and total fat mass (b) with stratified analysis of training duration < 8 represents studies with three sessions a week for less than 8 weeks. ≥8 represents studies with three sessions a week for 8 weeks or more. (1) and (2) represent the same study but different HIIT protocols. (a)–(d) represent the same study but different population characteristics. (§) and (§§) represent the same study but different analysis of abdominal fat mass. Other: studies that do not use three sessions per week

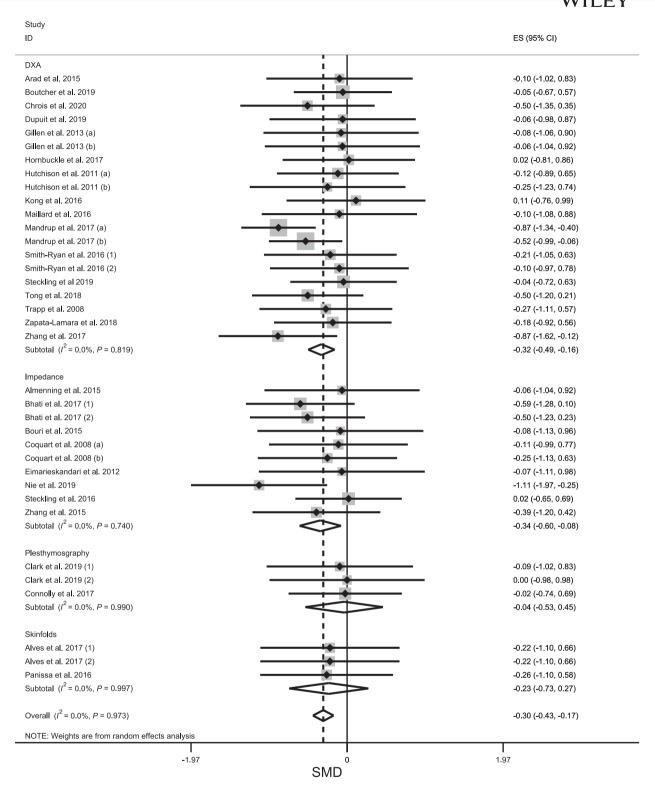


FIGURE 4 Continued

degree). In their meta-analysis, Batacan, Duncan, Dalbo, Tucker, and Fenning (2017) compared the effects of short-term HIIT (<12 weeks) and long-term HIIT (≥12 weeks). They concluded that at least 12 weeks of HIIT was necessary to improve body composition (reducing waist circumference and percentage of body fat) in overweight/obese

populations. In contrast with this study, our results showed that shorter interventions were able to generate body composition remodelling, at least in premenopausal women. The same conclusion is not possible in postmenopausal women since only the study of Mandrup et al., (2017) fulfilled our inclusion criteria of three training sessions a week.

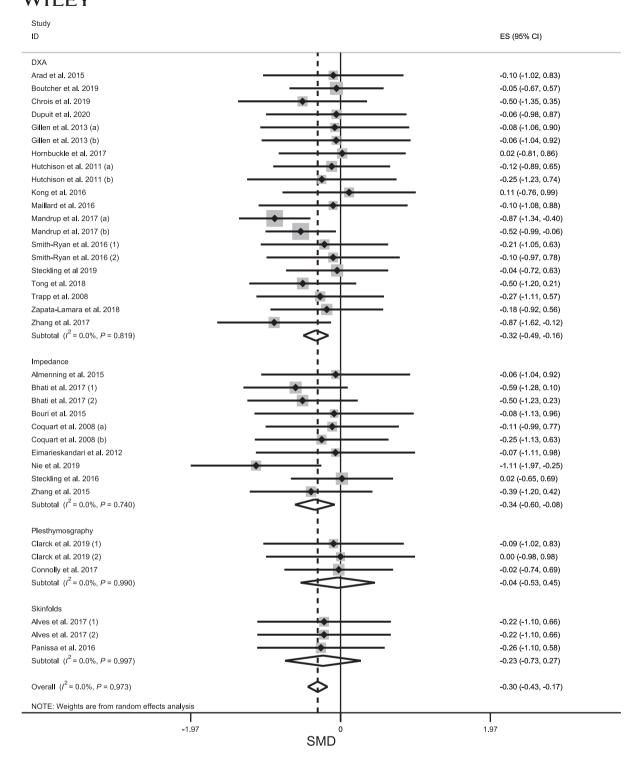


FIGURE 5 Forest plot for the effect of high-intensity interval training on total fat mass (a) and abdominal fat mass (b) with stratified analysis of body composition assessment. (1) and (2) represent the same study but different HIIT protocols. (a) and (b) represent the same study but different population characteristics. (§) and (§§) represent the same study but different analysis of abdominal fat mass

The last analysis of our review concerned the method used to assess body composition. DXA is a 'gold-standard' method for measuring total FM but is probably not the best for assessing abdominal and, more particularly, visceral FM (Bosch et al., 2015). While CT and MRI are probably the best methods to measure visceral adipose tissue (VAT) (Crabtree et al., 2019) evidence points to the potential usefulness of

DXA in providing accurate measurements of VAT in addition to total body composition determination in obese subjects (Bertin, Marcus, Ruiz, Eschard, & Leutenegger, 2000). However, its accuracy seems to be lower in individuals with high VAT levels (Bosch et al., 2015). Thus, as already shown by Shuster, Patlas, Pinthus, and Mourtzakis (2012), it is important to select the most suitable method for each parameter.

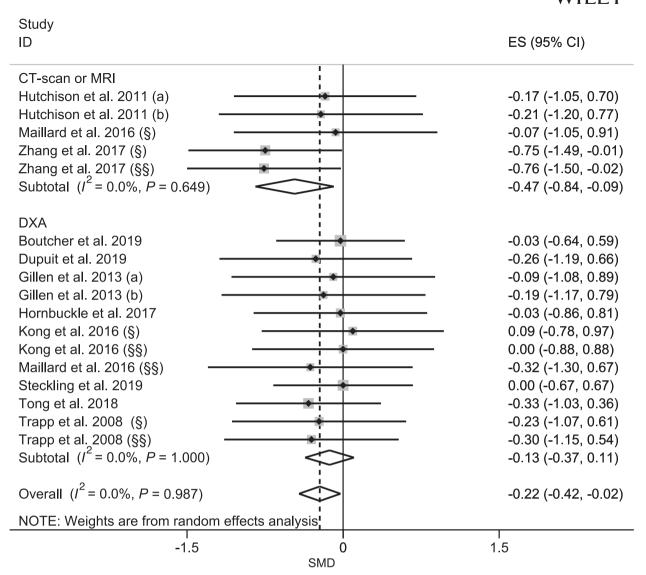


FIGURE 5 Continued

DXA, CT and MRI can be expensive and other methods for determining FM and FFM are often used, such as bioelectrical impedance and the skinfold test. In our analysis, total FM losses were only significant with DXA or bioelectrical impedance compared to plethysmography or skinfold measures. The loss of abdominal FM was only significant (or close to significance for visceral FM, P=0.055) when CT or MRI was used. Harmonization of methodological approaches and selecting the same anatomical regions for abdominal and visceral measurements could facilitate the interpretation of results and comparisons between studies.

4.1 | Limitations

The major limitations of this review are the lack of homogeneity of the populations in the studies analysed and the lack of details about the protocol used. For example, some publications included normalweight and overweight subjects, while others combined people with overweight and obesity. In our review, the mean BMI was used to classify each population since only very few studies involved a specific population with a BMI superior or inferior at 25 or 30. The use of BMI could also be a limitation since it does not reflect the percentage of FM or its localization, and its utilization seems less suitable with advancing age (De Lorenzo et al., 2020).

To compare the effects of the duration of HIIT, it was decided to analyse those studies with similar training frequencies, since a shorter programme duration can be offset by greater training frequency (and vice versa) (Stavrinou et al., 2018, 2019). In our meta-analysis, 33 of the 38 studies were carried out at a frequency of three times a week (i.e. 85%), because HIIT performs once or twice per week can limit metabolic adaptations (Dalleck et al., 2010; Kavaliauskas et al., 2017; Stavrinou et al., 2018, 2019).

In our review, we included bioelectrical impedance (n=14) and plethysmography (n=2) in the analysis for measuring FM changes despite the limits of these methods (Brodie et al., 1998; Kuriyan, 2018; Mulasi et al., 2015). While DXA is considered to be the best method

to determine whole-body FM, it has been shown that it does not accurately assess body composition in markedly obese women and that the level of hydration of the subjects can also alter total and/or regional FM measurements (Bredella et al., 2010; Barreira & Tseh 2020).

Another limitation concerns the inclusion of menopausal women. In certain studies, the groups of women classified as menopausal comprised only those who actually were menopausal, i.e. with at least 12 months of amenorrhoea. In others, however, only the mean age of the group was given with no indication of hormonal status. Some of these groups with a relatively young mean age probably included women over 40–45 years old, who in our analysis were considered as pre-menopausal. Finally, the lack of information regarding cycling programmes, such as the cycle ergometer used, revolutions per minute, resistance, watts and heart rate, and running protocols, such as speed, gradient treadmill and heart rate, make it difficult to compare two modalities or studies using the same modality.

5 | CONCLUSION

This is the first meta-analysis to investigate the effects of HIIT on adipose tissue alterations in the female population and to analyse separately these effects in pre- and postmenopausal women. When all the women were pooled together, our results confirmed that HIIT programmes significantly decreased body weight and total and abdominal FM. Both normal weight and overweight/obese women lost total FM after HIIT protocols whereas HIIT was only effective in decreasing abdominal FM in women with excess adiposity. When pre- and postmenopausal women were considered separately, the effect of HIIT on weight, total and abdominal FM was only significant before menopause. Owing to the very small number of publications involving postmenopausal women, it is difficult to reach any definitive conclusions and further studies are still needed. Nevertheless, oestrogen deficiency could play a role in these discrepancies. Finally, from a practical point of view, cycling HIIT seemed more effective than running, especially in postmenopausal women, and training interventions longer than 8 weeks comprising three sessions a week should be encouraged whatever the hormonal status and the adiposity level of the participants.

COMPETING INTERESTS

None of the authors declares competing financial interests.

AUTHOR CONTRIBUTIONS

D.M., M.F. and N.B. wrote the manuscript, participated in data acquisition and interpretation. P.B. carried out statistical analysis, M.L.M. and A.L.H. participated in manuscript reviewing/revising. All authors have read and approved the final version of this manuscript and agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All persons designated as authors qualify for authorship, and all those who qualify for authorship are listed.

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