Resveratrol and metabolic health in COPD

Clinical Nutrition 39 (2020) 2989–2997

Contents lists available at ScienceDirect

Clinical Nutrition

journal homepage: http://www.elsevier.com/locate/clnu

Randomized Control Trials

Resveratrol and metabolic health in COPD: A proof-of-concept randomized controlled trial

Rosanne JHCG. Beijers [a, *], Harry R. Gosker [a], Karin JC. Sanders [a], Chiel de Theije [a], Marco Kelders [a], Gerard Clarke [b], John F. Cryan [c], Bram van den Borst [a], Annemie MWJ. Schols [a]

[a] Department of Respiratory Medicine, NUTRIM School of Nutrition and Translational Research in Metabolism, Maastricht University Medical Centre+, Maastricht, the Netherlands
[b] APC Microbiome Ireland & Department of Psychiatry and Neurobehavioural Science, University College Cork, Cork, Ireland
[c] APC Microbiome Ireland & Department of Anatomy and Neuroscience, University College Cork, Cork, Ireland

ARTICLE INFO

Article history:
Received 29 May 2019
Accepted 7 January 2020

Keywords:
COPD
Resveratrol
Skeletal muscle
Adipose tissue
Body composition

SUMMARY

Background: Patients with COPD are often characterized by disturbed metabolic health which is reflected in altered body composition. Current studies in healthy subjects suggest that resveratrol improves metabolic health by enhancing muscle mitochondrial function and adipose tissue morphology. The primary objective was to investigate the effect of four weeks resveratrol supplementation on muscle mitochondrial function in patients with COPD. Secondary objectives were to investigate the effect of resveratrol on adipose tissue inflammatory and metabolic gene expression, systemic inflammation and body composition in patients with COPD.

Methods: In a double-blind randomized placebo-controlled proof-of-concept study, 21 COPD patients (FEV_1: 53 ± 15% predicted; age: 67 ± 9 years and BMI: 24.5 ± 3.3 kg/m^2) received resveratrol (150 mg/day) or placebo for four weeks. Before and after intervention, blood samples, quadriceps muscle and subcutaneous abdominal fat biopsies were obtained for metabolic and inflammatory profiling. Body composition was assessed by dual energy X-ray absorptiometry.

Results: Muscle mitochondrial biogenesis regulators AMPK, SIRT1 and PGC-1α as well as mitochondrial respiration, Oxphos complexes, oxidative enzyme activities and kynurenine aminotransferases were not improved by resveratrol. Plasma high-sensitive C-reactive protein and kynurenine did not change after resveratrol supplementation. Adipose tissue inflammatory markers were unaffected by resveratrol, while markers of glycolysis and lipolysis were significantly increased compared to placebo supplementation. Body weight decreased after resveratrol supplementation (resveratrol −0.95 ± 1.01 kg vs placebo −0.16 ± 0.66 kg, $p = 0.049$) due to a reduction in lean mass (resveratrol −1.79 ± 1.67 kg vs 0.37 ± 0.86 kg, $p = 0.026$).

Conclusion: We do not confirm previously reported positive effects of resveratrol on skeletal muscle mitochondrial function in patients with COPD, but show an unexpected decline in lean mass.

Clinical trial registry: Clinicaltrials.gov NCT02245932.

1. Introduction

Chronic obstructive pulmonary disease (COPD) is not only characterized by loss of lung function but also by reduced skeletal muscle mass and loss or redistribution of adipose tissue mass, which adversely affects metabolic health [1]. Decreased muscle metabolic health is well established in COPD [2,3]. Next to loss of muscle mass, intrinsic abnormalities include a shift from oxidative type I towards glycolytic type II muscle fibers and a reduced oxidative capacity [4,5]. Moreover, mitochondrial function is impaired: skeletal muscle mitochondria of patients with COPD rely more on the metabolically less-efficient complex-II driven mitochondrial respiration [6] and exhibit an increased reactive oxygen species production [7,8]. Muscle biopsies of patients with COPD also showed decreased protein levels of phosphorylated adenosine

* Corresponding author. P.O. Box 5800, 6202 AZ, Maastricht, the Netherlands. Fax: +31 43 3875051.
E-mail address: r.beijers@maastrichtuniversity.nl (R.JHCG. Beijers).

https://doi.org/10.1016/j.clnu.2020.01.002
0261-5614/

Abbreviations

AMPK	adenosine monophosphate-activated protein kinase
ATGL	adipose triglyceride lipase
CGI58	comparative gene identification-58
COPD	chronic obstructive pulmonary disease
CRP	C-reactive protein
DHEAS	dehydrposoandrosterone sulfate
DHR	dihydroresveratrol
FEV_1	forced expiratory volume in 1 s
HDL	high-density lipoprotein
HSL	home-sensitive lipase
KATs	kynurenine aminotransferases
LDL	low-density lipoprotein
PPAR	peroxisome proliferator-activated receptor
PGC-1α	PPAR gamma coactivator 1α
SIRT1	sirtuin 1
T2DM	type 2 diabetes mellitus
TFAM	mitochondrial transcription factor A

monophosphate-activated protein kinase (AMPK) and peroxisome proliferator-activated receptor (PPAR) gamma coactivator 1α (PGC-1α) [5,9,10]. In addition to skeletal muscle impairments in COPD, adipose tissue is lost [11] or redistributed towards an increase in ectopic fat [12]. Pro-inflammatory gene expression in subcutaneous adipose tissue of very severe COPD patients was significantly upregulated compared to moderate-severe COPD, despite a lower fat mass [13]. Systemic inflammation is also common in COPD [1]. Besides commonly employed markers of low-grade systemic inflammation, recently, increased plasma levels of the inflammatory marker kynurenine were reported in patients with COPD [14]. Interestingly, kynurenine can be cleared (by conversion into kynurenic acid) in skeletal muscle by kynurenine aminotransferases (KATs)1–4, which are under the control of PGC-1α [15]. This suggests that muscle impairments in COPD may further enhance systemic inflammatory load as well.

The natural polyphenol resveratrol (3,5,4′-trihydroxystilbene) is a bioactive nutrient, that has recently received a lot of attention based on promising multi-organ effects in experimental animal models including anti-inflammatory, anti-oxidant and cardioprotective effects as well as boosting effects on mitochondrial function [16]. However, to date clinical validation of cardioprotective effects in different target groups (i.e. obesity, type II diabetes, elderly) have been rather disappointing [16]. Resveratrol may nevertheless be an interesting pharmaconutrient for patients with COPD as it has been shown to activate Sirtuin 1 (SIRT1), directly or indirectly via the activation of AMPK, which subsequently activates PGC-1α, a master regulator of mitochondrial metabolism and biogenesis [17]. In addition, resveratrol supplementation in mice fed with a high-fat diet showed an inhibition of adipogenesis and inflammation and increased lipolysis in adipose tissue [18].

To our knowledge, no clinical study has been published investigating the systemic effects of resveratrol supplementation in patients with COPD. Therefore, the aim of this proof-of-concept study was to assess the effects of four weeks resveratrol supplementation on skeletal muscle mitochondrial function as primary outcome and on molecular signatures in muscle and adipose tissue biopsies, systemic inflammation and body composition as secondary outcome.

2. Methods

2.1. Study design and subjects

A randomized placebo-controlled double-blind clinical trial was conducted in clinically stable COPD patients. Patients were recruited via advertisements in local newspapers in the neighborhood of Maastricht, The Netherlands, between 2016 and 2017. Exclusion criteria were recent (<4 weeks) exacerbation that required oral steroids and/or hospitalization, diabetes mellitus (all types), active cardiovascular disease or a cardiovascular event (such as myocardial infarction, cerebrovascular haemorrhage/infarction) in the previous 6 months, recent major surgery, thyroid dysfunction, hepatic and renal disorders, current malignancy or central or obstructive sleep apnea. Furthermore, subjects using resveratrol containing dietary supplements or subjects with current alcohol consumption >20 g/day were excluded. All patients gave their written informed consent. The study was registered at clinicaltrials.gov (NCT02245932) and was approved by the Medical Ethics Committee from Maastricht University Medical Centre+ (MUMC+ [NL49391.068.14/MEC 14-3-016]).

2.2. Supplementation protocol

Subjects received either resveratrol (150 mg/day trans-resveratrol [99.9%]; resVida; provided by DSM Nutritional Products Ltd.) or placebo for four weeks (28 days). This dose is much higher than the dose of resveratrol that can be ingested though the diet [19]. The resveratrol dosage and duration of supplementation was based on several previous studies in healthy obese, type II diabetes mellitus patients and first degree relatives of patients with type II diabetes mellitus in which an improvement in muscle mitochondrial function was found [20–22]. Randomization was carried out by an independent researcher and the randomization scheme was controlled for current smoking and gender using the minimization method [23,24]. Both subjects and researchers were blinded for the treatments until the end of the study analysis. Patients were instructed to take the first supplement after the measurements on the first test-day and the last supplement in the evening before the last test-day. On the last test-day, patients returned unused capsules in order to check for compliance. Patients were instructed to maintain their usual dietary intake and physical activity pattern and use of medication and (if applicable) other supplements during the intervention period. Supplement and medication intake was checked by self-report and physical activity pattern was checked by measuring physical activity levels during the 7 days before the test-days using a dual-axis GT1M accelerometer (ActiGraph™, LLC, Fort Walton Beach, FL, USA), as was previously described [5]. To check compliance, resveratrol metabolites (trans-resveratrol and dihydroresveratrol (DHR)) were measured by mass spectrometry in plasma as was previously described [22]. Furthermore, general safety parameters (creatinine, urea, sodium, potassium, Y-glutamyl transferase, alanine aminotransferase, aspartate aminotransferase and alkaline phosphatase) were analyzed.

2.3. Measurements

See the supplementary material for a detailed description of the measurements and the analysis.

A muscle biopsy of the *vastus lateralis* muscle of the dominant leg was taken under local anesthesia (2% lidocaine) during fasting conditions according to the technique by Bergström et al. [25]. A portion of the muscle tissue was directly frozen in liquid nitrogen and stored at −80 °C until analyzing protein expression by Western

blot, gene expression by qPCR and mitochondrial DNA copy number. Another portion (~50 mg) was immediately placed in ice-cold preservation medium (BIOPS) for determination of *ex vivo* muscle mitochondrial respiration using an oxygraph (OROBOROS Instruments, Innsbruck, Austria) as previously described [26]. Briefly, 5 mg saponin was solved in 1 mL BIOPS. Subsequently, 50 mL of this saponin/BIOPS solution was added to 5 mL BIOPS, in which muscle fibers were incubated for 30 min. After permeabilization, muscle fibers were transferred to the respiration chamber of the oxygraph to measure oxygen consumption. Two substrate inhibition protocols were used to measure oxidative phosphorylation. In the first protocol, substrates were added to the respiration chamber in the order: malate (M), octanoyl carnitine (O), ADP, glutamate (G), succinate (S), cytochrome C and fluoro-carboyl cyanide phenylhydrazone (FCCP). The same substrates in the absence of octanoyl carnitine were used in the second protocol. One patient was excluded from muscle biopsy analyses because no muscle biopsy was available and one patient was excluded from Western blot analysis because no tissue was left for analysis.

A para-umbilically subcutaneous adipose tissue biopsy was obtained during fasting conditions through needle biopsy. This biopsy was snap-frozen in liquid nitrogen and stored at −80 °C until analyzing gene expression by qPCR. One patient was excluded from these analyses because no adipose tissue biopsy was available.

Plasma kynurenine, kynurenic acid and tryptophan were measured by high-performance liquid chromatography as described previously [27]. The kynurenine to tryptophan ratio was calculated as a measure of the inflammatory status. The kynurenic acid to kynurenine ratio was calculated as a measure of the conversion of kynurenine in the skeletal muscle.

Body composition was assessed by Dual Energy X-ray Absorptiometry (DXA, Hologic, Discovery A, QDR Series, Bedford, MA, USA). Furthermore, cardiometabolic risk parameters including glucose, insulin, low-density and high-density lipoprotein (LDL and HDL) cholesterol, total cholesterol, triglycerides and high sensitive C-reactive protein were measured in fasting blood samples (see supplementary methods). Blood pressure was measured and metabolic syndrome was determined according to the NCEP ATP definition [28]. Lung function was assessed using spirometry (SpiroPerfect™, Welch Allyn, Delft, the Netherlands) at the first test-day.

2.4. Statistical analyses

Sample size calculation was based on previous observed 10–20% improvements in mitochondrial respiration after resveratrol supplementation [20–22,29]. In total, 20 subjects were required to achieve 80% power, with an assumed treatment difference of 0.435 mmol/min/kg (15%) after 4 weeks resveratrol and an assumed standard deviation of 0.3 mmol/min/kg [7].

Statistical analyses were performed blinded using Statistical Package for the Social Sciences (SPSS version 22 for Windows, SPSS Inc. Chicago, IL). Results are presented as mean ± standard deviation (SD) when normally distributed and as median (interquartile range) if not normally distributed, unless indicated otherwise. All data was checked on influential outliers which were subsequently excluded. Differences between groups at baseline were compared using Student t-test for continuous variables, X^2-test for categorical variables and Kruskal Wallis test for continuous variables with skewed distributions. Data were analyzed using a two-way repeated measures ANOVA with treatment (resveratrol and placebo) and test-day (pre and post) as within subject factors. In case of significant interactions (treatment × time), within group differences were analyzed using a paired sampled *t*-test. A *p*-value <0.05 was considered statistically significant.

Table 1
Baseline characteristics.

	Placebo (n = 10)	Resveratrol (n = 11)	*p*-value
Age, y	65.3 ± 9.1	67.8 ± 9.0	0.532
Males, n (%)	5 (50.0)	7 (63.6)	0.528
Smoking status			
Current smokers, n (%)	2 (20.0)	3 (27.3)	0.696
Former smokers, n (%)	8 (80.0)	8 (72.7)	
FEV_1/FVC, %	51.1 ± 9.1	44.8 ± 12.6	0.205
FEV_1, %pred	60 ± 13	48 ± 14	0.067
FVC, %pred	91 ± 15	84 ± 12	0.255
BMI, kg/m^2	24.9 ± 3.0	24.1 ± 3.7	0.594
Lean mass, kg	45.1 ± 7.4	46.3 ± 8.4	0.729
Fat mass, kg	24.9 ± 7.9	21.7 ± 7.0	0.330
Metabolic syndrome, n (%)	2 (20.0)	3 (27.3)	0.696
Total physical activity, counts/min	221 ± 102	195 ± 89	0.532
Total steps per day	5385 ± 2372	4299 ± 2111	0.281

Data were analyzed using independent sample t-test or X^2-square test and data are presented as mean ± standard deviation or n (%). BMI; body mass index; FEV_1, forced expiratory volume in 1 s; FVC, forced vital capacity.

3. Results

Twenty-three subjects were randomized of whom 21 completed the study (Supplementary Fig. 1). Baseline characteristics of these 21 subjects are summarized in Table 1: both groups were comparable in age, gender and smoking status and no significant differences were observed in lung function, body composition and metabolic health determined by prevalence and components of the metabolic syndrome (for individual components see Supplementary Table 5). Furthermore, both groups were comparable in physical activity level (Table 1), which they maintained during the intervention period (data not shown).

3.1. Compliance

Supplements were well-tolerated, no adverse events and no effects on safety parameters were reported (Supplement Table 4). Compliance was confirmed by the number of supplements returned (96.6% compliance) and by analysis of plasma levels of resveratrol and DHR. At baseline, both resveratrol and DHR were below detection. After the intervention period both metabolites were present at the expected levels in the resveratrol group (resveratrol 469.2 ± 218.0 ng/mL and DHR 915.5 ± 635.1 ng/mL), while both compounds were undetectable in the placebo group.

3.2. Skeletal muscle mitochondrial metabolism

Muscle biopsies were obtained from 20 subjects (n = 10 resveratrol, n = 10 placebo). *Ex vivo* muscle mitochondrial respiration revealed no significant effects of resveratrol compared to placebo. ADP-stimulated (state 3) respiration on a lipid substrate (malate + octanoyl-carnitine, MO) and upon parallel electron input to both complex I and II (MOG and MOGS) was not changed after resveratrol supplementation (Fig. 1B, C). Similar data were found on state 3 mitochondrial respiration upon complex I- and II-linked substrates in the absence of a lipid-derived substrate (MG and MGS) (Fig. 1D, E) and on maximal FCCP-induced uncoupled respiration (Fig. 1F). Citrate synthase and hydroxyl-acyl CoA dehydrogenase enzyme activities were also not affected by resveratrol compared to placebo (Fig. 1G, H). Moreover, mitochondrial DNA copy number, an index of mitochondrial mass, did not change significantly by resveratrol compared to placebo (Δ38.8 ± 573.6

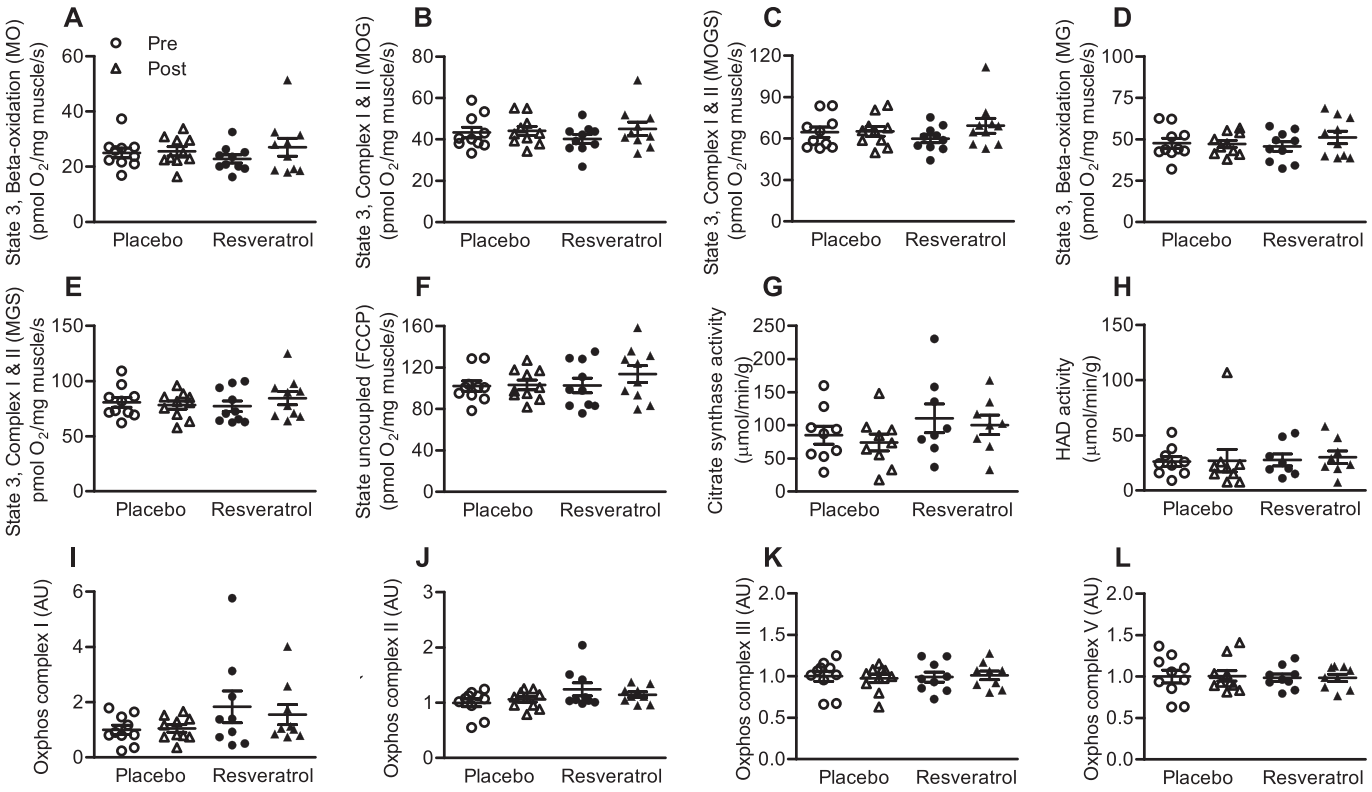

Fig. 1. Muscle mitochondrial respiration and Oxphos complex protein levels before (white bars) and after (black bars) resveratrol (n = 10) and placebo supplementation (n = 10). Oxidative capacity by means of *ex vivo* respirometry on skeletal muscle tissue: (A–C) ADP stimulated respiration (state 3) upon a lipid-like substrate and upon parallel electron input into complex I and II; (D–F) ADP stimulated respiration (state 3) in the absence of a lipid-derived substrate and upon parallel electron input into complex I and II; (G) Citrate synthase activity; (H) Hydroxy acyl-CoA dehydrogenase (HAD) activity; (I–L) Protein content of the individual complexes of the electron transport chain analyzed by Western Blot (resveratrol n = 9 and placebo n = 10). Values are arbitrary units relative to baseline measurement of the placebo group, unless indicated otherwise. Data are presented as individual data points and mean ± SEM. AU, Arbitrary Units; FCCP; carbonyl cyanide p-trifluoromethoxyphenylhydrazone; G, glutamate; M, malate; O, octanoyl-carnitine; Oxphos, oxidative phosphorylation; S, succinate. Data were analyzed using a two-way repeated measures ANOVA with treatment and test-day as within subject factors. None of the time × treatment interactions were statistically significant.

vs −30.5 ± 377.6 AU after resveratrol vs placebo, $p = 0.754$). Similar results were obtained if respiration rates were corrected for mitochondrial DNA copy number or citrate synthase (data not shown). Protein content of the individual Oxphos complexes (Fig. 1I–L), mitochondrial biogenesis regulators AMPK, SIRT1 and PGC-1α (Fig. 2) as well as gene expression of these markers (Supplementary Fig. 2) revealed no significant differences after resveratrol supplementation compared to placebo.

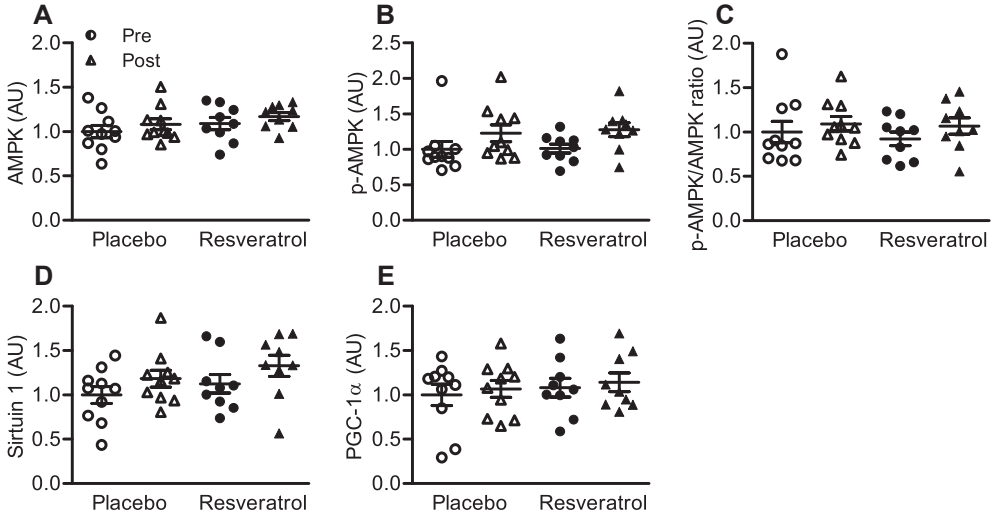

Fig. 2. Muscle protein levels of mitochondrial biogenesis regulators before (white bars) and after (black bars) resveratrol (n = 9) and placebo supplementation (n = 10). (A) AMPK; (B) phosphorylated AMPK; (C) Ratio of phosphorylated AMPK to total AMPK protein expression; (D) Sirtuin 1 protein expression; (E) PGC-1α protein expression. Values are arbitrary units relative to baseline (pre) measurement of the placebo group. AU, Arbitrary Units. Data are presented as individual data points and mean ± SEM. Data were analyzed using a two-way repeated measures ANOVA with treatment and test-day as within subject factors. None of the time × treatment interactions were statistically significant.

Table 2
Plasma high-sensitive C-reactive protein, kynurenine, kynurenic acid and tryptophan levels.

	Placebo (n = 10)		Resveratrol (n = 11)		*p*-value
	Before	After	Before	After	
Hs-CRP, mg/L	2.3 (1.4, 3.2)	2.4 (1.9, 6.0)	2.9 (1.8, 7.8)	2.1 (1.6, 4.7)	0.114
KYN, ng/mL	635 (498, 726)	636 (544, 779)	597 (417, 679)	535 (406, 612)	0.132
Trp, ng/mL	11,384 (10,493, 12,342)	12,406 (11,268, 13,486)[a]	12,578 (9529, 14,781)	11,362 (8879, 13,445)	**0.006**
KYNA, ng/mL	25.2 (12.1, 47.5)	16.3 (12.0, 24.1)	6.4 (3.4, 30.1)	13.8 (2.9, 34.2)	0.132
KYN/Trp	0.06 (0.04, 0.06)	0.05 (0.04, 0.06)	0.05 (0.04, 0.06)	0.04 (0.04, 0.05)	0.468
KYN/KYNA	19.7 (14.7, 73.2)	36.0 (30.9, 50.4)	98.6 (13.9, 205.9)	29.4 (18.6, 176.4)	0.099

Data were analyzed using Kruskal Wallis test for continuous variables with skewed distributions and results are presented as median (interquartile range).
Hs-CRP, High-sensitive C-reactive protein, KYN, kynurenine; KYNA, kynurenic acid; Trp, tryptophan.
p-values < 0.05 are indicated in bold.
[a] Significantly different from baseline. p-value shows time × treatment comparison.

3.3. Systemic inflammation and kynurenine pathway

Baseline plasma levels of high-sensitive CRP, tryptophan, kynurenine and kynurenic acid were not different between both intervention groups and did not correlate with diseases severity (data not shown). Plasma levels of high-sensitive CRP were not affected by resveratrol supplementation. Plasma levels of tryptophan significantly increased after placebo supplementation, which was significantly different from resveratrol supplementation (Table 2). Plasma kynurenine and kynurenic acid as well as the ratios between kynurenine, tryptophan and kynurenic acid did not significantly change after resveratrol supplementation. Protein and gene expression levels were not significantly affected by resveratrol supplementation (Fig. 3).

3.4. Adipose tissue inflammatory and metabolic gene expression

Adipose tissue biopsies were obtained from 20 subjects (n = 10 resveratrol, n = 10 placebo). Gene expression levels of markers of inflammation and macrophages were not significantly different after resveratrol supplementation compared to placebo (Fig. 4). Changes in markers of glycolysis and lipolysis were significantly different between the resveratrol and placebo group, in which markers increased after resveratrol supplementation while they decreased after placebo supplementation (Fig. 5). Gene expression levels of adipokines, mitochondrial markers and markers of hypoxia were not significantly affected by resveratrol supplementation (Supplementary Fig. 3).

3.5. Body composition

Body weight significantly decreased after resveratrol supplementation compared to placebo supplementation (−0.95 ± 1.01 kg vs −0.16 ± 0.66 kg, $p = 0.049$) due to a significant decrease in lean mass (Fig. 6). Changes in lean mass significantly correlated with changes in body weight (r = 0.441, $p = 0.046$). Body fat mass was not significantly altered after resveratrol supplementation compared to placebo. The changes in lean mass were not related to changes in protein and gene expression levels of anabolic and catabolic markers in skeletal muscle (Supplementary Fig. 4).

4. Discussion

To our knowledge, this is the first clinical study investigating the effects of resveratrol supplementation in patients with COPD. We report no changes in skeletal muscle mitochondrial respiration

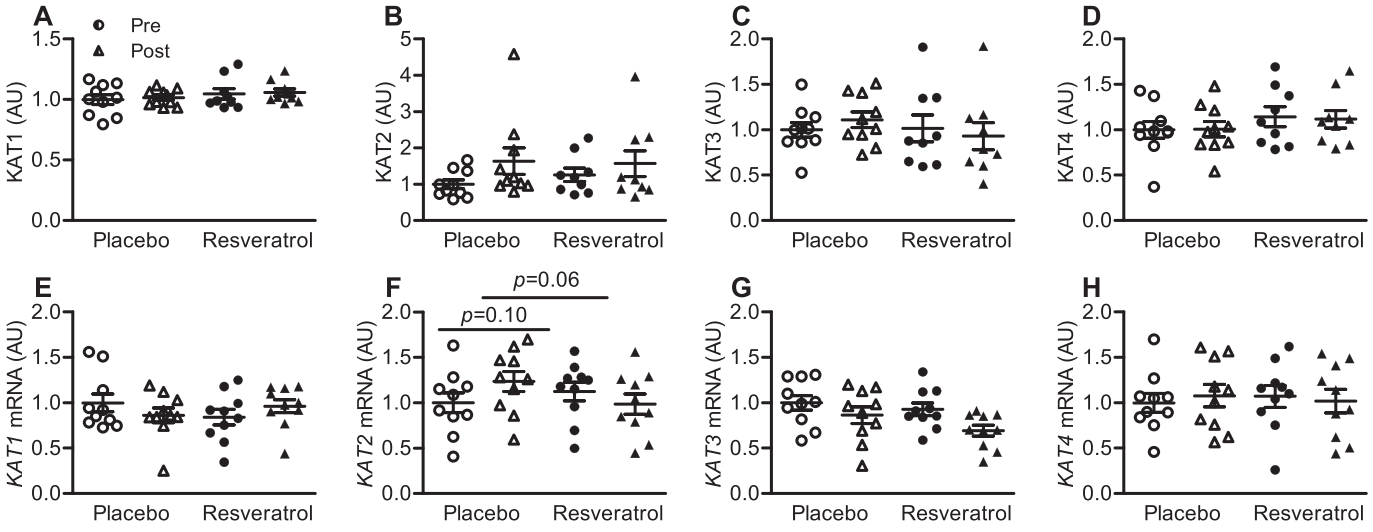

Fig. 3. Protein and gene expression levels of kynurenine aminotransferases (KAT) 1–4, before (white bars) and after (black bars) resveratrol (n = 10) and placebo supplementation (n = 10). Protein expression of (A) KAT1; (B) KAT2; (C) KAT3; (D) KAT4; by Western Blot (resveratrol n = 9 and placebo n = 10) and gene expression of (E) KAT1; (B) KAT2; (C) KAT3; (D) KAT4 by qPCR. Values are arbitrary units relative to baseline (pre) measurement of the placebo group. AU, Arbitrary Units. Data are presented as individual data points and mean ± SEM. Data were analyzed using a two-way repeated measures ANOVA with treatment and test-day as within subject factors. In case of significant interactions, within group differences were analyzed using a paired sampled *t*-test. Only *p*-values for statistically significant time × treatment interactions are shown.

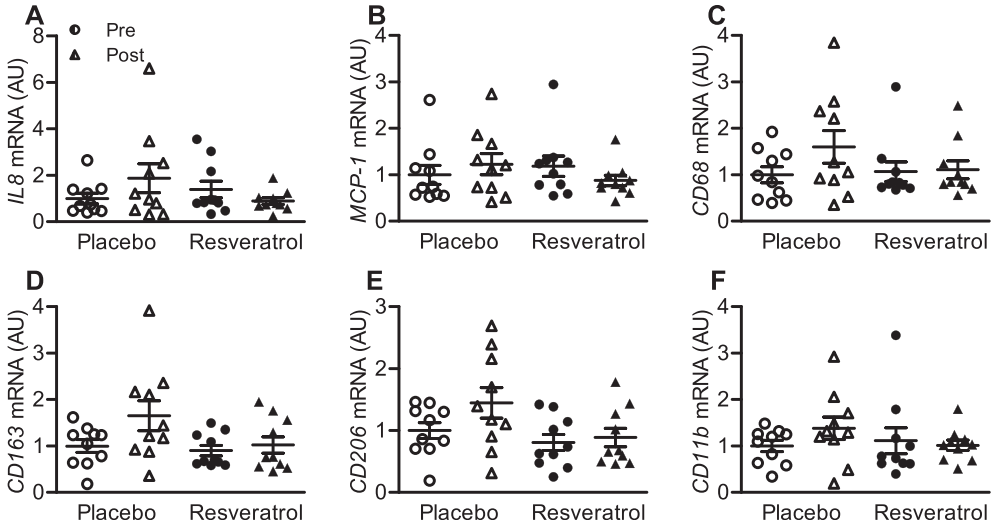

Fig. 4. Adipose tissue gene expression levels of markers of inflammation and macrophages, before (white bars) and after (black bars) resveratrol (n = 10) and placebo supplementation (n = 10). Gene expression of (A) Interleukin-8 (IL-8); (B) Monocyte chemotactic protein-1 (MCP-1); (C) CD68; (D) CD163; (E) CD206; (F) CD11b. AU, Arbitrary Units. Data are presented as individual data points and mean ± SEM. Data were analyzed using a two-way repeated measures ANOVA with treatment and test-day as within subject factors. None of the time × treatment interactions were statistically significant.

after resveratrol supplementation and in line with this, no changes in muscle AMPK, SIRT1 or PGC-1α were found. Systemic and adipose tissue inflammatory markers were unaffected after resveratrol supplementation but a differential response in adipose tissue glycolytic and lipolytic marker expression was observed between the groups, in which expression seemed to decrease after placebo supplementation while it maintained or even increased after resveratrol supplementation. A significant between group difference in body weight was observed, due to a reduction in lean mass after resveratrol supplementation.

Resveratrol is a SIRT1 activator, directly or indirectly via AMPK, that subsequently activates PGC-1α [17]. In the current study, SIRT1 and phosphorylated AMPK tended to increase after resveratrol supplementation, however this was not significantly different from the placebo group and this tendency was also not found for the other mitochondrial biogenesis markers downstream of SIRT1. Furthermore, muscle mitochondrial respiration did not significantly increase after resveratrol supplementation compared to placebo. These findings are not in agreement with previous studies in overweight and obese subjects, T2DM patients and relatives of T2DM patients [20–22,29], in which improved muscle mitochondrial respiration on the electron input of both complexes I and II after resveratrol supplementation was reported [20,22,29]. These improvements were also observed in the absence of increased AMPK and PGC-1α levels [21]. Three of these studies used a crossover design, but the same dose of resveratrol for a comparable duration of 30 days in respectively n = 13, n = 17 and n = 11 subjects [20–22].

Plasma levels of tryptophan significantly increased after placebo supplementation, which was significantly different from resveratrol supplementation. This differential response corresponds to

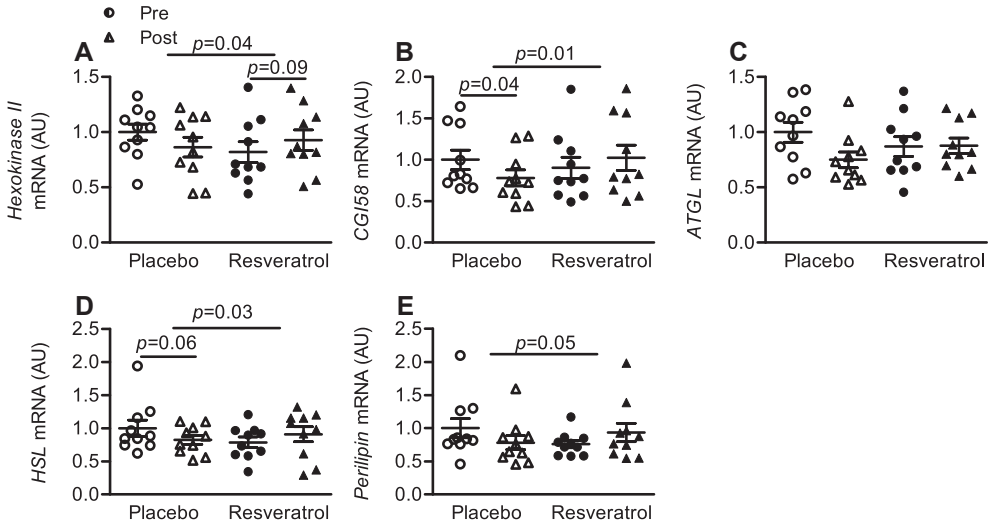

Fig. 5. Adipose tissue gene expression levels of markers of glycolysis and lipolysis, before (white bars) and after (black bars) resveratrol (n = 10) and placebo supplementation (n = 10). Gene expression of (A) Hexokinase II; (B) Comparative gene identification-58 (CGI-58); (C) Adipose triglyceride lipase (ATGL); (D) Home-sensitive lipase (HSL). (E) Perilipin. AU, Arbitrary Units. Data are presented as individual data points and mean ± SEM. Data were analyzed using a two-way repeated measures ANOVA with treatment and test-day as within subject factors. In case of significant interactions, within group differences were analyzed using a paired sampled *t*-test. Only *p*-values for statistically significant time × treatment interactions are shown.

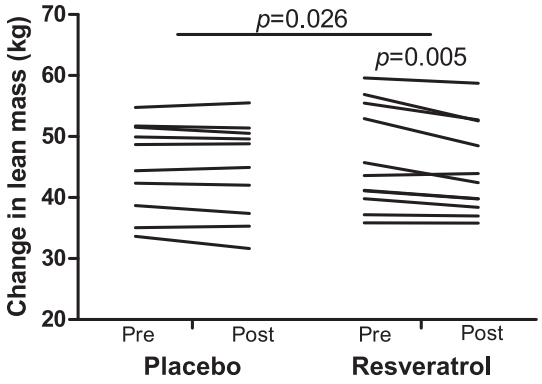

Fig. 6. Changes in lean mass after placebo (n = 10) and resveratrol (n = 11) supplementation. Data were analyzed using a two-way repeated measures ANOVA with treatment and test-day as within subject factors. Within group differences were analyzed using a paired sampled *t*-test.

previous findings in which healthy volunteers ingested 5 g of resveratrol and showed a significant decrease in plasma tryptophan levels [30]. However, the latter study also found slightly elevated kynurenine levels after resveratrol supplementation, which resulted in a significantly increased kynurenine to tryptophan ratio. These features of the kynurenine pathway were not affected in the current study. Since we also did not find an effect of resveratrol on the expression of PGC-1α in skeletal muscle and subsequently not on the KATs, the lack of this effect may not be completely surprising. Change in tryptophan might have an effect on mood as tryptophan is a precursor of serotonin synthesis [31,32]. Assessing mood however was outside the scope of this study, but might be relevant in future clinical trials.

A striking observation was a significant within and between group decrease in body weight and specifically in lean body mass after resveratrol. A recent comprehensive metabolomics analysis in blood, urine, fat and skeletal muscle showed that the most pronounced effect of four months resveratrol in middle aged men with metabolic syndrome was a change in steroid hormones across all four matrices, in which sulfated androgen precursors were reduced in blood, adipose tissue, and muscle tissue and increased in urine [33]. More specifically, in skeletal muscle the sulfated steroid metabolites dehydroisoandrosterone sulfate (DHEAS), epiandrosterone sulfate, androsterone sulfate and 4-adrosten3β, and 17β-diol disulfate 1 were significantly decreased. This may be relevant for (sarcopenic) patients with COPD as previous research showed that serum DHEAS levels were significantly lower in patients with low compared to normal mid-thigh cross-sectional area [34]. Ratios between the catabolic cytokine interleukin-1 and the catabolic hormone cortisol to the anabolic hormone DHEAS were also significantly lower in COPD patients with low mid-thigh cross-sectional area compared to controls. An imbalance between anabolic and catabolic systemic triggers may affect muscle protein turnover and drive muscle wasting in COPD. It could be speculated that resveratrol stimulates catabolic signaling in muscle. However, in the current study markers of anabolic and catabolic processes (Supplementary Fig. 4) were not changed in muscle biopsies after resveratrol supplementation. These findings are in contrast to observations from experimental models. In C2C12 and L6 myotubes or mice treated with catabolic inflammatory or hormonal triggers (tumor necrosis factor (TNF)-α or dexamethasone), expression of the atrogenes atrogin-1 and MuRF1 was increased [35–37], but prevented when resveratrol was simultaneously administered. A recent meta-analysis confirmed a decrease in body weight albeit after longer-term intervention [38]. The fact that the currently reported weight decrease was due to a decrease in lean mass was unexpected and seems to contrast with limited available data in healthy (obese) subjects [39,40]. In view of the enormous popularity of resveratrol use in the US (annual growth of 5.8% since 2013 [41]), our data call for further investigation in COPD.

Subcutaneous adipose tissue biopsies were primarily obtained to analyze inflammatory markers, which were unaffected by resveratrol supplementation in line with an unaltered systemic inflammatory profile. Secondary and similar to the muscle biopsies, we analyzed metabolic markers of oxidative metabolism in these adipose tissue biopsies, which showed significant differences in gene expression levels of some markers of lipolysis and glycolysis. A previous study investigated the effect of resveratrol on lipolysis in human and murine adipocytes and adipose tissue of mice and specifically assessed the involvement of adipose triglyceride lipase (ATGL) and home-sensitive lipase (HSL) [42]. This study reported that resveratrol increased lipolysis mainly through enhancing ATGL expression. We did not find an effect of resveratrol on ATGL expression but we did find significant between group differences in expression of lipolysis markers comparative gene identification-58 (CGI58), HSL and perilipin. The current study furthermore showed increased hexokinase II gene expression after resveratrol supplementation. More research is needed regarding the effect of resveratrol on adipose tissue oxidative metabolism in COPD.

Several limitations of the current study need to be considered. One could argue that the absence of a healthy control group to confirm a decreased mitochondrial function in COPD, is a limitation of the current study. However, the aim of this RCT was based on consistent previous research showing impaired muscle mitochondrial function in patients with COPD and comparable airflow obstruction [5,9,43]. Despite randomization, lung function tended to be lower in the resveratrol group. However, this was not statistically significant and airflow obstruction did not correlate with any changes in mitochondrial function and body composition (data not shown). Moreover, physical activity level was comparable between the groups. Another limitation is that dietary intake was not evaluated in this study, which next to elevated energy requirements could have contributed to the observed body weight changes in this study. However, one might expect that a resveratrol-induced effect on energy balance might primarily affect fat tissue instead of the observed decreased lean mass. Bioavailability of resveratrol is low [44], but in the current study, the dose and duration were based on previous studies showing beneficial effects of resveratrol on mitochondrial function [20–22]. Since these studies did find significant improvements in muscle mitochondrial function after 4 weeks resveratrol supplementation of 150 mg/day, it is believed that the 4-week intervention period in the current study would be sufficient to find relevant changes in mitochondrial function. Moreover, the total plasma resveratrol levels were even higher in the current study compared to these previous studies (183–412 ng/mL).

In conclusion, the present study in patients with COPD showed no beneficial effect of four weeks resveratrol supplementation on skeletal muscle mitochondrial function, systemic and adipose tissue inflammation, but seemed to affect adipose tissue oxidative metabolism. Remarkably and unexpected, four weeks of resveratrol decreased body weight due to decreased lean mass, which calls for further investigation, particularly if resveratrol is being considered as a candidate intervention targeting lung injury in COPD.

Funding sources

This study was financially supported by Lung Foundation Netherlands (grant: 3.4.12.023) and by the JPI HDHL project

“Ambriosiac” by ZonMW (project number: 529051006). ResVida and placebo capsules were provided by DSM Nutritional Products Ltd.

Author contributions

Designed research: RB, HG, BB, AS; conducted research: RB, KS, BB; analyzed data or performed statistical analysis: RB, HG, CT, MK, GC, JC; wrote paper: RB, HG, AS; had primary responsibility for final content: RB, HG, KS, CT, MK, GC, JC, BB, AS.

Conflict of interest

The authors have no conflict of interest to declare.

Acknowledgments

The authors thank DSM Nutritional Products Ltd. for providing the resVida and placebo capsules and for performing the resveratrol and DHR analysis.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.clnu.2020.01.002.

References

[1] Vogelmeier CF, Criner GJ, Martinez FJ, Anzueto A, Barnes PJ, Bourbeau J, et al. Global strategy for the diagnosis, management, and prevention of chronic obstructive lung disease 2017 report. GOLD executive summary. Am J Respir Crit Care Med 2017;195(5):557–82.
[2] Rabinovich RA, Vilaro J. Structural and functional changes of peripheral muscles in chronic obstructive pulmonary disease patients. Curr Opin Pulm Med 2010;16(2):123–33.
[3] Patel AR, Hurst JR. Extrapulmonary comorbidities in chronic obstructive pulmonary disease: state of the art. Expert Rev Respir Med 2011;5(5):647–62.
[4] Gosker HR, Zeegers MP, Wouters EF, Schols AM. Muscle fibre type shifting in the vastus lateralis of patients with COPD is associated with disease severity: a systematic review and meta-analysis. Thorax 2007;62(11):944–9.
[5] van den Borst B, Slot IG, Hellwig VA, Vosse BA, Kelders MC, Barreiro E, et al. Loss of quadriceps muscle oxidative phenotype and decreased endurance in patients with mild-to-moderate COPD. J Appl Physiol (1985) 2013;114(9):1319–28.
[6] Gifford JR, Trinity JD, Layec G, Garten RS, Park SY, Rossman MJ, et al. Quadriceps exercise intolerance in patients with chronic obstructive pulmonary disease: the potential role of altered skeletal muscle mitochondrial respiration. J Appl Physiol (1985) 2015;119(8):882–8.
[7] Puente-Maestu L, Perez-Parra J, Godoy R, Moreno N, Tejedor A, Gonzalez-Aragoneses F, et al. Abnormal mitochondrial function in locomotor and respiratory muscles of COPD patients. Eur Respir J 2009;33(5):1045–52.
[8] Puente-Maestu L, Tejedor A, Lazaro A, de Miguel J, Alvarez-Sala L, Gonzalez-Aragoneses F, et al. Site of mitochondrial reactive oxygen species production in skeletal muscle of chronic obstructive pulmonary disease and its relationship with exercise oxidative stress. Am J Respir Cell Mol Biol 2012;47(3):358–62.
[9] Natanek SA, Gosker HR, Slot IG, Marsh GS, Hopkinson NS, Moxham J, et al. Pathways associated with reduced quadriceps oxidative fibres and endurance in COPD. Eur Respir J 2013;41(6):1275–83.
[10] Remels AH, Gosker HR, van der Velden J, Langen RC, Schols AM. Systemic inflammation and skeletal muscle dysfunction in chronic obstructive pulmonary disease: state of the art and novel insights in regulation of muscle plasticity. Clin Chest Med 2007;28(3):537–52. vi.
[11] Schols AM, Broekhuizen R, Weling-Scheepers CA, Wouters EF. Body composition and mortality in chronic obstructive pulmonary disease. Am J Clin Nutr 2005;82(1):53–9.
[12] Maddocks M, Shrikrishna D, Vitoriano S, Natanek SA, Tanner RJ, Hart N, et al. Skeletal muscle adiposity is associated with physical activity, exercise capacity and fibre shift in COPD. Eur Respir J 2014;44(5):1188–98.
[13] Tkacova R, Ukropec J, Skyba P, Ukropcova B, Pobeha P, Kurdiova T, et al. Increased adipose tissue expression of proinflammatory CD40, MKK4 and JNK in patients with very severe chronic obstructive pulmonary disease. Respiration 2011;81(5):386–93.
[14] Zinellu A, Fois AG, Zinellu E, Sotgiu E, Sotgia S, Arru D, et al. Increased kynurenine plasma concentrations and kynurenine-tryptophan ratio in mild-to-moderate chronic obstructive pulmonary disease patients. Biomark Med 2018;12(3):229–37.
[15] Agudelo LZ, Femenia T, Orhan F, Porsmyr-Palmertz M, Goiny M, Martinez-Redondo V, et al. Skeletal muscle PGC-1alpha1 modulates kynurenine metabolism and mediates resilience to stress-induced depression. Cell 2014;159(1):33–45.
[16] Beijers R, Gosker HR, Schols A. Resveratrol for patients with chronic obstructive pulmonary disease: hype or hope? Curr Opin Clin Nutr Metab Care 2018;21(2):138–44.
[17] de Ligt M, Timmers S, Schrauwen P. Resveratrol and obesity: can resveratrol relieve metabolic disturbances? Biochim Biophys Acta 2015;1852(6):1137–44.
[18] Kim S, Jin Y, Choi Y, Park T. Resveratrol exerts anti-obesity effects via mechanisms involving down-regulation of adipogenic and inflammatory processes in mice. Biochem Pharmacol 2011;81(11):1343–51.
[19] Baur JA, Sinclair DA. Therapeutic potential of resveratrol: the in vivo evidence. Nat Rev Drug Discov 2006;5(6):493–506.
[20] de Ligt M, Bruls YMH, Hansen J, Habets MF, Havekes B, Nascimento EBM, et al. Resveratrol improves ex vivo mitochondrial function but does not affect insulin sensitivity or brown adipose tissue in first degree relatives of patients with type 2 diabetes. Mol Metab 2018;12:39–47.
[21] Timmers S, de Ligt M, Phielix E, van de Weijer T, Hansen J, Moonen-Kornips E, et al. Resveratrol as add-on therapy in subjects with well-controlled type 2 diabetes: a randomized controlled trial. Diabetes Care 2016;39(12):2211–7.
[22] Timmers S, Konings E, Bilet L, Houtkooper RH, van de Weijer T, Goossens GH, et al. Calorie restriction-like effects of 30 days of resveratrol supplementation on energy metabolism and metabolic profile in obese humans. Cell Metabol 2011;14(5):612–22.
[23] Han B, Enas NH, McEntegart D. Randomization by minimization for unbalanced treatment allocation. Stat Med 2009;28(27):3329–46.
[24] Pocock SJ, Simon R. Sequential treatment assignment with balancing for prognostic factors in the controlled clinical trial. Biometrics 1975;31(1):103–15.
[25] Bergstrom J, Hermansen L, Hultman E, Saltin B. Diet, muscle glycogen and physical performance. Acta Physiol Scand 1967;71(2):140–50.
[26] Phielix E, Schrauwen-Hinderling VB, Mensink M, Lenaers E, Meex R, Hoeks J, et al. Lower intrinsic ADP-stimulated mitochondrial respiration underlies in vivo mitochondrial dysfunction in muscle of male type 2 diabetic patients. Diabetes 2008;57(11):2943–9.
[27] Clarke G, Fitzgerald P, Cryan JF, Cassidy EM, Quigley EM, Dinan TG. Tryptophan degradation in irritable bowel syndrome: evidence of indoleamine 2,3-dioxygenase activation in a male cohort. BMC Gastroenterol 2009;9:6.
[28] Grundy SM, Cleeman JI, Daniels SR, Donato KA, Eckel RH, Franklin BA, et al. Diagnosis and management of the metabolic syndrome: an American heart association/National heart, lung, and blood institute scientific statement. Circulation 2005;112(17):2735–52.
[29] Most J, Timmers S, Warnke I, Jocken JW, van Boekschoten M, de Groot P, et al. Combined epigallocatechin-3-gallate and resveratrol supplementation for 12 wk increases mitochondrial capacity and fat oxidation, but not insulin sensitivity, in obese humans: a randomized controlled trial. Am J Clin Nutr 2016;104(1):215–27.
[30] Gualdoni GA, Fuchs D, Zlabinger GJ, Gostner JM. Resveratrol intake enhances indoleamine-2,3-dioxygenase activity in humans. Pharmacol Rep 2016;68(5):1065–8.
[31] Cervenka I, Agudelo LZ, Ruas JL. Kynurenines: tryptophan's metabolites in exercise, inflammation, and mental health. Science 2017;357(6349).
[32] Richard DM, Dawes MA, Mathias CW, Acheson A, Hill-Kapturczak N, Dougherty DM, et al. Basic metabolic functions, behavioral research and therapeutic indications. Int J Tryptophan Res 2009;2:45–60.
[33] Korsholm AS, Kjaer TN, Ornstrup MJ, Pedersen SB. Comprehensive metabolomic analysis in blood, urine, fat, and muscle in men with metabolic syndrome: a randomized, placebo-controlled clinical trial on the effects of resveratrol after four months' treatment. Int J Mol Sci 2017;18(3).
[34] Debigare R, Marquis K, Cote CH, Tremblay RR, Michaud A, LeBlanc P, et al. Catabolic/anabolic balance and muscle wasting in patients with COPD. Chest 2003;124(1):83–9.
[35] Alamdari N, Aversa Z, Castillero E, Gurav A, Petkova V, Tizio S, et al. Resveratrol prevents dexamethasone-induced expression of the muscle atrophy-related ubiquitin ligases atrogin-1 and MuRF1 in cultured myotubes through a SIRT1-dependent mechanism. Biochem Biophys Res Commun 2012;417(1):528–33.
[36] Liu J, Peng Y, Wang X, Fan Y, Qin C, Shi L, et al. Mitochondrial dysfunction launches dexamethasone-induced skeletal muscle atrophy via AMPK/FOXO3 signaling. Mol Pharm 2016;13(1):73–84.
[37] Wang DT, Yin Y, Yang YJ, Lv PJ, Shi Y, Lu L, et al. Resveratrol prevents TNF-alpha-induced muscle atrophy via regulation of Akt/mTOR/FoxO1 signaling in C2C12 myotubes. Int Immunopharmacol 2014;19(2):206–13.
[38] Mousavi SM, Milajerdi A, Sheikhi A, Kord-Varkaneh H, Feinle-Bisset C, Larijani B, et al. Resveratrol supplementation significantly influences obesity measures: a systematic review and dose-response meta-analysis of randomized controlled trials. Obes Rev 2019;20(3):487–98.
[39] Arzola-Paniagua MA, Garcia-Salgado Lopez ER, Calvo-Vargas CG, Guevara-Cruz M. Efficacy of an orlistat-resveratrol combination for weight loss in subjects with obesity: a randomized controlled trial. Obesity (Silver Spring) 2016;24(7):1454–63.

[40] Kjaer TN, Ornstrup MJ, Poulsen MM, Stodkilde-Jorgensen H, Jessen N, Jorgensen JOL, et al. No beneficial effects of resveratrol on the metabolic syndrome: a randomized placebo-controlled clinical trial. J Clin Endocrinol Metab 2017;102(5):1642–51.
[41] Young SN. The effect of raising and lowering tryptophan levels on human mood and social behaviour. Philos Trans R Soc Lond 2013;368(1615): 20110375.
[42] Lasa A, Schweiger M, Kotzbeck P, Churruca I, Simon E, Zechner R, et al. Resveratrol regulates lipolysis via adipose triglyceride lipase. J Nutr Biochem 2012;23(4):379–84.
[43] Remels AH, Schrauwen P, Broekhuizen R, Willems J, Kersten S, Gosker HR, et al. Peroxisome proliferator-activated receptor expression is reduced in skeletal muscle in COPD. Eur Respir J 2007;30(2):245–52.
[44] Walle T. Bioavailability of resveratrol. Ann N Y Acad Sci 2011;1215:9–15.