**Name of Journal: *World Journal of Hepatology***

**ESPS Manuscript NO: 26954**

**Manuscript Type: Original Article**

***Clinical Trials Study***

**Independent effects of diet and exercise training on fat oxidation in non-alcoholic fatty liver disease**

Croci I *et al.* Diet and exercise interventions for NAFLD

**Ilaria Croci, Nuala M Byrne, Veronique S Chachay, Andrew P Hills, Andrew D Clouston, Trisha M O’Moore-Sullivan, Johannes B Prins, Graeme A Macdonald, Ingrid J Hickman**

**Ilaria Croci, Veronique S Chachay, Ingrid J Hickman,** Translational Research Institute, the University of Queensland Diamantina Institute, Woolloongabba 4102, Australia

**Ilaria Croci, Veronique S Chachay,** School of Human Movement and Nutrition Sciences, University of Queensland, St. Lucia 4072, Australia

**Nuala M Byrne,** Bond Institute of Health and Sport, Bond University, Robina 4229, Australia

**Nuala M Byrne,** Institute of Health and Biomedical Innovation, Queensland University of Technology, Kelvin Grove 4059, Australia

**Andrew P Hills, Trisha M O’Moore-Sullivan, Johannes B Prins, Ingrid J Hickman,**Mater Research Institute University of Queensland, Brisbane 4101, Australia

**Andrew P Hills,** School of Health Sciences, University of Tasmania, Launceston 7250, Australia

**Andrew D Clouston, Graeme A Macdonald,**School of Medicine, the University of Queensland, Brisbane 4102, Australia

**Graeme A Macdonald,**Department of Gastroenterology and Hepatology, Princess Alexandra Hospital, Woolloongabba 4102, Australia

**Graeme A Macdonald,** Translational Research Institute, Woolloongabba 4102, Australia

 **Ingrid J Hickman,** Department of Nutrition and Dietetics, Princess Alexandra Hospital, Woolloongabba 4102, Australia

**Author contributions:** Croci I wrote the paper; Croci I, Byrne NM, Chachay VS, Hills AP, Clouston AD, O’Moore-Sullivan TM, Prins JB, Macdonald GA and Hickman IJ designed the research; Croci I, Chachay VS, O’Moore-Sullivan TM, Macdonald GA and Hickman IJ collected data; Croci I, Hickman IJ and Clouston AD analyzed the data; Hickman IJ obtained funding; all authors approved the manuscript.

**Support by** The National Health and Medical Research Council of Australia; and the Lions Medical Research Foundation.

**Institutional review board statement:** The study was approved by the Human Research Ethics Committees of the Princess Alexandra Hospital and the University of Queensland.

**Clinical trial registration statement:** This registration policy applies to prospective, randomized, controlled trials only.

**Informed consent statement:** All study participants, or their legal guardian, provided informed written consent prior to study enrollment.

**Conflict-of-interest statement:** The authors have no conflicts of interest to disclose.

**Data sharing statement:** No additional data are available.

**Open-Access:** This article is an open-access article which selected by an in-house editor and fully peer-reviewed by external reviewers. It distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: http://creativecommons.org/licenses/by-nc/4.0/

**Manuscript source:** Invited manuscript

**Correspondence to: Ilaria Croci, PhD,** School of Human Movement and Nutrition Sciences, University of Queensland**,** Blair Drive,St Lucia 4072, Australia.ilaria.croci@uqconnect.edu.au

**Telephone:** +61-7-33656851

**Fax:** +61-7-33656877

**Received:** April 30, 2016

**Peer-review started:** May 3, 2016

**First decision:** June 17, 2016

**Revised:** July 21, 2016

**Accepted:** August 17, 2016

**Article in press:**

**Published online:**

**Abstract**

**AIM:** To investigate the independent effects of 6-mo of dietary energy restriction or exercise training on whole-body and hepatic fat oxidation of patients with non-alcoholic fatty liver disease (NAFLD).

**METHODS:** Participants were randomised into either circuit exercise training (EX; *n* = 13; 3 h/wk without changes in dietary habits), or dietary ER without changes in structured physical activity (ER; *n* = 8).Respiratory quotient (RQ) and whole-body fat oxidation rates (Fatox) were determined by indirect calorimetry under basal, insulin-stimulated and exercise conditions. Severity of disease and steatosis was determined by liver histology; hepatic Fatox was estimated from plasma β-hydroxybutyrate concentrations; cardiorespiratory fitness was expressed as VO2peak. Complete-case analysis was performed (EX: *n* = 10; ER: *n* = 6).

**RESULTS:** Hepatic steatosis and NAFLD activity score decreased with ER but not with EX. β-hydroxybutyrate concentrations increased significantly in response to ER (0.08 ± 0.02 *vs* 0.12 ± 0.04, *P* = 0.03) but remained unchanged in response to EX (0.10 ± 0.03 *vs* 0.11 ± 0.07, *P* = 0.39). Basal RQ decreased (*P* = 0.05) in response to EX, while this change was not significant after ER (*P* = 0.38). VO2peak (*P* < 0.001) and maximal Fatox during aerobic exercise (*P* = 0.03) improved with EX but not with ER (*P* > 0.05). The increase in β-hydroxybutyrate concentrations was correlated with the reduction in hepatic steatosis (*r* = -0.56, *P* = 0.04).

**CONCLUSION:** ER and EX lead to specific benefits on fat metabolism of patients with NAFLD. Increased hepatic Fatox in response to ER could be one mechanism through which the ER group achieved reduction in steatosis.

**Key words:** Non-alcoholic steatohepatitis; Steatosis; Fat and carbohydrate oxidation; Exercise; Fitness; Beta-hydroxybutyrate; Ketone bodies; Fatty acid oxidation

**© The Author(s) 2016.** Published by Baishideng Publishing Group Inc. All rights reserved.

**Core tip:** We investigated hepatic fat oxidation and whole-body substrate oxidation under basal, insulin-stimulated and exercise conditions before and after 6 mo of circuit exercise training (EX) or dietary energy restriction (ER) in patients with non-alcoholic fatty liver disease. ER increased β-hydroxybutyrate concentrations (a marker of hepatic fat oxidation) and reduced severity of steatosis, but did not change substrate oxidation rates during acute exercise. EX improved substrate oxidation under basal, insulin-stimulated and exercise conditions, but not β-hydroxybutyrate concentrations and severity of disease. Increase in β-hydroxybutyrate was associated with decrease in hepatic steatosis and this could be one mechanism through which the ER group achieved reduction in steatosis.

Croci I, Byrne NM, Chachay VS, Hills AP, Clouston AD, O’Moore-Sullivan TM, Prins JB, Macdonald GA, Hickman IJ. Independent effects of diet and exercise training on fat oxidation in non-alcoholic fatty liver disease. *World J Hepatol* 2016; In press

**INTRODUCTION**

Non-alcoholic fatty liver disease (NAFLD) is the most prevalent liver disease in industrialized countries and its prevalence is increasing globally[1]. The term NAFLD describes a range of liver damage ranging from simple steatosis to non-alcoholic steatohepatitis (NASH) and cirrhosis that occur in the absence of hazardous alcohol consumption. NAFLD is linked with obesity, visceral adiposity, physical inactivity, insulin resistance[2], and genetic predisposition[3]. Intrahepatic triglycerides (TGs) (steatosis) accumulate when the sum of *de novo* hepatic fatty acid synthesis rate and hepatic fatty acid uptake rate is greater than those of TG export and hepatic fat oxidation[4]. In a recent cross-sectional study we have shown that overweight patients with NAFLD do not adequately adapt fuel oxidation to fuel availability, with reduced fat oxidation rates (Fatox) in resting and fasting conditions, a reduced suppression of Fatox after insulin stimulation and a lower increase in Fatox during exercise compared to lean controls[5]. Further, we observed that patients with NAFLD had reduced hepatic Fatox, as measured by plasma β-hydroxybutyrate, when compared to lean controls.

Lifestyle interventions consisting of diet (improved diet quality with or without energy restriction) or diet in conjunction with exercise training are currently the most commonly advocated therapies for NAFLD management[6-8]. Limited research has assessed the effect of a lifestyle intervention in NAFLD on whole-body Fatox. Hallsworth *et al*[9] showed that eight weeks of resistance training without weight loss did not change substrate oxidation rates in the basal state (resting and fasting) but increased Fatox during aerobic exercise. However, substrate oxidation during exercise was assessed at a single intensity and at the same absolute intensity pre and post intervention (50% of the pre-intervention VO2peak). Therefore, assessment of maximal rate of Fatox (MFO) and the intensity at which it occurs (Fatmax) was not possible, and participants likely were assessed at a lower relative intensity post-intervention (due to improvedVO2peak). Gaining a deeper understanding of substrate metabolism during exercise is of interest because the full body metabolic demands are higher and potential alterations not observable at rest may become apparent.

The effect of different treatment options for NAFLD on hepatic Fatox is also unclear. In response to dietary ER, little information is available. A study in which 18 patients with NAFLD underwent 2 wk of dietary ER reported increased plasma β-hydroxybutyrate concentrations (indicating increased hepatic Fatox), and this was correlated with reduction in steatosis[10]. This is in agreement with findings in animal models showing that an increase in hepatic Fatox leads to a reduction in hepatic steatosis[11,12]. However, whether a similar response is seen in response to a longer dietary intervention, with the assessment being performed in energy balance (as opposed to energy deficit), needs to be established. Furthermore, the effect of an exercise training program on plasma β-hydroxybutyrate concentrations is unknown[13]. Understanding the independent effect of energy restriction and exercise training on whole-body Fatox and hepatic Fatox in patients with NAFLD can contribute to elucidate how these interventions impact on the disease and could lead to more specific guidelines for NAFLD management.

Improvement in cardiorespiratory fitness (CRF) is a key endpoint in exercise training interventions. Cross-sectional evidence shows that lower levels of physical activity and CRF correlate with more severe hepatic injury on histology and greater steatosis[14-17]. However, the relationship between change in CRF measured with a graded exercise test and change in steatosis (measured quantitatively) has not been explored longitudinally in NAFLD[18,19]. Investigating the associations between changes in markers of CRF, substrate oxidation, and histological, metabolic and biochemical features of NAFLD in response to exercise can help understand the mechanisms through which exercise may benefit features of NAFLD.

This study aimed to investigate changes in hepatic Fatox and in whole-body substrate oxidation rates under basal, insulin-stimulated and exercise conditions, in patients with NAFLD who completed either six months of dietary energy restriction or circuit exercise training. The second aim was to assess whether changes in CRF, whole-body fat and hepatic Fatox were associated with changes in hepatic steatosis.

**MATERIALS AND METHODS**

### Participants

Overweight patients with NAFLD (diagnosed on liver biopsy) participated in the study (*n* = 21). Exclusion criteria included: Type 2 diabetes, cirrhosis, decompensated liver disease, presence of other causes of liver disease, and daily ethanol consumption > 20 g in females or > 40 g in males. The study was approved by the local Human Research Ethics Committees (Princess Alexandra Hospital and University of Queensland, Australia). All participants provided informed written consent. The randomized controlled clinical trial was registered with the Australian and New Zealand Clinical Trials Registry (<http://www.anzctr.org.au>). The registration identification number is ACTRN12612001087842.

### General design

Participants were randomised into either a dietary energy restriction intervention (ER; *n* = 8) or an exercise training intervention (EX; *n* = 13). A consort diagram describing the flow of patients through the randomised controlled trial is presented in Figure 1. Outcome measures were assessed prior to randomisation (pre-intervention) and after 6 mo of intervention. At both time-points participants undertook three testing sessions within a 7-d period. Patients had stable body weight for at least 2 wk before the post intervention testing.

During the first testing session, body composition was assessed by dual-energy X-ray absorptiometry. The second session involved a hyperinsulinaemic-euglycaemic clamp with indirect calorimetry measurements to assess substrate oxidation rates under basal and insulin-stimulated conditions. This session also involved clinical assessments, including blood pressure and anthropometry. During the third testing session, indirect calorimetry measurement was performed during a graded exercise test on an ergocycle to determine substrate oxidation rate and CRF (as measured by VO2peak). The second and third sessions were conducted in the morning after an overnight fast. Both ER and EX groups were instructed not to change exercise and physical activity patterns throughout the intervention and this was monitored with accelerometers at three time points during the intervention.

The primary outcomes of the trial were hepatic steatosis and IR and have been published elsewhere[20]. The present manuscript focuses on secondary outcome measures including plasma β-hydroxybutyrate concentrations, and whole-body Fatox under basal, insulin-stimulated and exercise conditions. The flow of participants for the present analysis is presented in the Consort Diagram in Figure 1. Complete-case analysis, including 10 EX and 6 ER participants, was performed.

### Exercise training intervention (EX)

#### EX, as previously detailed[20], involved 3 sessions per week of circuit exercise training during 6 mo without dietary restriction. The aim was to improve CRF, muscle strength and body composition without significant body weight loss. EX was selected based on preliminary research conducted in our laboratory[21].

Training intensity was fixed at 50% of 1-RM for the entire duration of the training program; 1-RM was reassessed monthly to account for strength adaptations. The training volume was progressively increased from one circuit (12 min) in week 1 to five circuits (60 min) in week 11; and then remained constant at five circuits from week 11 until the end of the intervention. Each circuit comprised 12 light resistance exercises covering the major muscle groups. The training program consisted of alternating 30 s exercise intervals and 30 s rest periods. Pneumatic resistance training machines were employed (Ab Hur Oy, Kokkola, Finland). All training sessions were supervised by an exercise physiologist.

### Energy restriction intervention (ER)

#### ER involved a weight loss program under the guidance of a dietitian. Patients attended weekly face-to-face appointments for 16 wk and were provided with an individualised dietary prescription with the aim of 5%-10% of body weight loss within 16 wk. This was followed by an 8-wk period aimed at body weight maintenance, with fortnightly reviews with the dietitian. The target macronutrient composition was 40% carbohydrate, 20% protein and 40% fat (< 10% saturated fat). Recommendations included choosing foods that are low in saturated fats; avoiding micronutrient-poor/energy-dense food options; avoiding added sugar; and aiming for regular meal patterns. Weekly weight and waist measures, and 24-h diet recalls encouraged adherence and self-monitoring.

***Histological analysis of liver biopsy***

Liver biopsy specimens were analysed as previously detailed[5,20]. The severity of liver injury was determined with the NAFLD activity score (NAS)[22] and the criteria described by Brunt[23]. Using conventional histologic criteria[24], a diagnosis of NASH or steatosis alone was made.

***Body composition***

Body composition assessments including determination of fat-free mass (FFM) and fat mass by dual-energy X-ray absorptiometry. Subcutaneous abdominal fat and visceral abdominal fat were assessed by computed tomography as previously described[25].

***Insulin sensitivity***

Insulin sensitivity was assessed with the hyperinsulinemic-euglycemic clamp technique[26], as we previously detailed[20]. Briefly, primed insulin was infused at a rate of 1 mU/kg per minute throughout the procedure (2 h), and a 25% glucose solution was infused at a variable rate to maintain euglycemia[26]. The glucose infusion rate in the steady state of the hyperinsulinemic-euglycemic clamp (M-value) corresponded to the whole-body glucose disposal rate.

***Biochemical analysis***

Biochemical analyses were performed as previously described[5,20]. Plasma β-hydroxybutyrate concentrations, an index of hepatic ketogenesis[27-30], were measured with an enzymatic assay (Stanbio, Boerne, TX, CV 2.2%).

***Exercise testing***

Maximal aerobic power and substrate utilization were assessed with a graded exercise test on an ergocycle. Testing comprised a sub-maximal phase to determine Fatox and CHOox at multiple intensities (with workload increments occurring every 5 min), and a maximal phase to assess peak oxygen consumption (VO2peak) (increments every min). The testing protocol adopted has been described in detail in a previous publication[5].

***Indirect calorimetry measurements***

Indirect calorimetry measurements (TrueOne 2400 Metabolic Measurement System, Parvo Medics, UT) were conducted in three physiological states (basal, insulin-stimulated and exercise). Whole-body Fatox and CHOox were calculated using stoichiometric equations, with the assumption that the urinary nitrogen excretion rate was negligible[31]. The methodological approach adopted has been previously described in detail[5].

Fatox rates during exercise were estimated from respiratory gazes averaged over the last minute of each exercise stage. Then, the stage at which maximal fat oxidation (MFO) was achieved was determined, and the corresponding intensity was identified (Fatmax)[32]. ΔRQ represented the RQ change from basal to hyperinsulineamic state (RQ in the insulin-stimulated condition minus basal RQ).

Testing sessions involving indirect calorimetry measurements were conducted in the morning after a 10-12 h overnight fast and under standardised conditions[5]. Standardisation of pre-test conditions was in line with previous studies[32-40].

***Daily physical activity***

Daily physical activity was quantified with RT3 accelerometers Activity Monitor, 2003, Stayhealthy, Incorporated, Monrovia, CA, United States) worn for 7 consecutive days at 0, 3 and 6 mo, as previously described[20].

### Statistical analysis

A secondary analysis of outcomes from a larger clinical trial was performed. Independent *t*-tests were used to compare the pre-intervention (baseline) characteristics between groups (ER *vs* EX). Paired Student *t*-tests were used to compare within group outcome measures pre and post intervention. Wilcoxon matched-pair signed rank test was used if samples were not normally distributed. Correlation analyses were performed using Pearson’s correlation coefficient or Spearman’s non-parametric rank correlation coefficient. As outlined in the consort diagram (Figure 1), complete-case analysis was performed. Complete-case analysis was deemed more suitable than intention to treat analysis given that the aim of this study was to study mechanisms of benefit of the two interventions. Statistical analysis was performed with SPSS 17.0 (SPSS, Chicago, IL, United States) and Graph Pad Prism 5.0 (GraphPad Software, San Diego, CA, United States). Data are expressed as mean ± standard deviation (SD) or median and range. For all statistical analyses, the level of significance was set at *P* < 0.05. Statistical methods used in this study were reviewed a biostatistician.

## RESULTS

### Characteristics of study groups

Two patients from each arm (*n* = 4) did not complete the study due to time constraints. One participant (*n* = 1) from the EX group was excluded from analysis due to significant weight loss at 6 mo (-13.3% body weight, which cannot be achieved with the type and volume of exercise prescribed as part of this exercise intervention). Data analysis (complete-case analysis) was thus performed on 10 participants from the EX and 6 participants from the ER groups (see the Consort Diagram presented in Figure 1). There were no significant differences between pre-intervention patients’ characteristics of completers and non-completers. Compliance with both interventions was good. The ER group achieved an average weight loss of 9.7% ± 4.6%, and the EX group attendance to the exercise sessions was greater than 90% with no significant weight loss. As per protocol, usual daily time spent on low, moderate and high intensity physical activity did not change in either group (*P* > 0.05). ER and EX interventions were well tolerated by participants with no adverse events reported.

Characteristics of the EX and ER groups are presented in Table 1. At baseline, the prevalence of NASH was not different between ER and EX groups (67% *vs* 80%, *P* = 0.64). Primary results of the randomised controlled trial are reported elsewhere[20]. Briefly, in the ER group steatosis and the NAS decreased significantly, while in the EX group neither steatosis nor NAS decreased significantly. Skeletal muscle insulin resistance (M-value) improved significantly in response to EX, while it did not improve in patients from the ER group.

### Substrate oxidation under basal conditions

Total energy expenditure in resting and fasted conditions (basal) did not significantly change in response to both interventions (*P* > 0.05). However, with the EX intervention the relative contribution of fat and CHO to energy expenditure changed: The RQ and the CHOox decreased (by 30%, *P* = 0.02), while Fatox tended to increase (Table 2). With the ER intervention, the same direction of change as for EX was seen, however statistical significance was not reached. In the whole-group, the pre-post intervention change in basal RQ was not associated with the pre-post intervention changes in steatosis (*r* = 0.05, *P* = 0.88) or NAS (*P* = 0.35).

### β-hydroxybutyrate concentrations

As shown in Figure 2, basal plasma β-hydroxybutyrate concentrations, increased significantly in response to ER (0.08 ± 0.02 *vs* 0.12 ± 0.04, *P* = 0.03) but remained unchanged in response to EX (0.10 ± 0.03 *vs* 0.11 ± 0.07, *P* = 0.39). This result (unchanged β-hydroxybutyrate concentrations in response to EX) was confirmed also when the analysis was performed excluding the outlier (0.09 ± 0.03 *vs* 0.09 ± 0.03, *P* = 0.87) (Figure 2). In the combined cohort including participants from both groups, there was a negative association between pre-post intervention changes in β-hydroxybutyrate and in hepatic steatosis (*r* = -0.56, *P* = 0.04) (Figure 3). This relationship persisted after controlling for changes in body weight (*r* = -0.67, *P* = 0.02) and percentage body weight (*r* = -0.56, *P* = 0.05).

### Substrate oxidation under insulin-stimulated conditions

Hyperinsulinaemic concentrations were reached by both groups at both times points (ER, 79.0 ± 31.5 mU/L *vs* 80.0 ± 21.5 mU/L; EX, 83.0 ± 0.5 mU/L *vs* 78.1 ± 18.0 mU/L; all *P* > 0.05). The effect of the two interventions on substrate oxidation in insulin-stimulated conditions is presented in Table 3. Post-intervention, the EX group tended to increase the insulin-stimulated suppression of Fatox compared with pre-intervention (-0.24 ± 0.36 mg/kgFFM per minute *vs* -0.55 ± 0.35 mg/kgFFM per minute, *P* = 0.06). The ER group displayed a similar response, however statistical significance was not reached. In the pooled group, the pre-post intervention increase in ΔRQ (change in RQ from the basal to the insulin-stimulated state) was not correlated with the change in the severity of steatosis (*r* = 0.28, *P* = 0.28) or NAS (*P* = 0.31).

### Substrate oxidation during exercise

VO2peak and MFO improved significantly (by 18% and 71%, respectively) in response to EX but did not change in the ER group (Table 4 and Figure 4). Fatmax increased by 72% in response to EX when expressed in absolute terms (45 ± 20 *vs* 76 ± 46 Watts, *P* = 0.03), whereas it remained unchanged after both interventions when expressed in relative terms (%VO2peak). Within the EX group, the increase in VO2peak (mL/kgFFM per minute) was correlated with the increase in ΔRQ (*r* = 0.73, *P* = 0.02) and the reduction in systolic blood pressure (*r* = -0.81, *P* = 0.01). The improvement in VO2peak was not related with the change in steatosis (*r* = 0.14, *P* = 0.73), NAS (*P*=0.40) or basal RQ (*r* = -0.18, *P* = 0.62). Similarly, the change in MFO was not related to changes in hepatic steatosis (*r* = 0.03, *P* = 0.91), or changes in NAS (*P* = 0.63).

## DISCUSSION

ER and EX are standard interventions for the management of obesity and related comorbidities, including NAFLD. ER induced weight loss, reduced hepatic steatosis, increased β-hydroxybutyrate concentrations (a marker of hepatic Fatox) but did not lead to changes in substrate oxidation rates tested during an acute exercise session. EX lead to improvements in CRF and in substrate oxidation rates under basal, insulin stimulated and exercise conditions. However, this dose of circuit EX did not lead to improvements in hepatic Fatox or hepatic steatosis. In the combined cohort, the reduction in hepatic steatosis was associated with increased β-hydroxybutyrate concentrations.

A novel finding from this study was that ER and EX interventions had different effects on β-hydroxybutyrate concentrations in patients with NAFLD. In response to ER, the increase in β-hydroxybutyrate (product of the oxidation pathway) was accompanied by the trend for a decrease in the very low-density lipoprotein (product of the esterification pathway), despite no change in free fatty acids concentrations. These are favourable changes given that pre-intervention patients with NAFLD showed lower β-hydroxybutyrate and higher very low-density lipoprotein compared to healthy controls[5]. These changes may suggest that the ER intervention lead to a change in hepatic fatty acid partitioning, with free fatty acids being more directed towards oxidation than towards esterification[41]. Increase in hepatic Fatox could be a mechanism through which the ER group achieved reduction in steatosis. Accordingly, it was shown in animal models that interventions that increase hepatic Fatox lead to a reduction in hepatic steatosis[11,12].

In contrast, β-hydroxybutyrate concentrations remained unaltered in response to EX. This observation is valuable because, as highlighted in a recent review, no information is available on the chronic effects of EX on β-hydroxybutyrate concentrations[13]. Results from the present study do not confirm findings from rodent models, which showed that chronic EX increased hepatic Fatox[42] and that the shift from an active to a sedentary lifestyle reduced hepatic Fatox[43]. Future studies assessing the effects of different training prescriptions (volume, intensity, frequency, duration) and the optimal type of exercise (aerobic exercise *vs* circuit EX *vs* resistance training) on hepatic lipid metabolism are warranted. Inclusion of genetic and molecular parameters in future investigations might provide insights on the mechanisms responsible for the inter-individual variability observed in response to the treatments.

The effect of the two interventions on MFO was different: It markedly increased in response to EX, while it remained unchanged in response to ER. The improvement in MFO in the exercise group could be attributable to increased mitochondrial content, increased oxidative capacity and improved transport of free fatty acids across muscle and mitochondrial membranes[44-47]. Such changes likely were not achieved in response to ER[48]. To our knowledge, this was the first study comparing the effect of two types of lifestyle intervention (*i.e.*, ER and EX) on MFO in patients with NAFLD. It was also the first study to assess Fatmax and MFO in response to circuit exercise training. The improvement observed in MFO was consistent with previous studies conducted in other populations: higher whole-body Fatox during exercise was observed in response to a moderate intensity aerobic training program conducted in obese males[49], and in response to high-intensity aerobic training[50] or resistance exercise training[51] programs conducted in healthy individuals. Overall, the improvement in MFO in response to EX and lack of change in response to ER are in agreement with findings from a recent cross-sectional study showing that substrate oxidation rates during exercise are correlated with CRF but not with body weight or percentage body fat[52].

Another observation from the present study was that EX improved whole-body substrate oxidation rates in resting and insulin-stimulated conditions (greater Fatox in basal conditions and greater increase in CHOox in response to insulin stimulation). The increased basal whole-body Fatox observed in response to EX treatment is in agreement with studies conducted in obese patients[53,54]. On the other hand, no change was observed by the only other study which investigated whole-body fat oxidation in response to exercise training in NAFLD. The different outcome compared to the present study could be explained by the shorter duration of the intervention (8 wk) and the different baseline characteristics of the study population (less severe NAFLD)[9]. In response to ER, there appeared to be a change towards a greater proportion of basal energy expenditure derived from Fatox, however statistical significance was not achieved due to the small sample size. These results are in line with other dietary interventions involving high-fat diets with carbohydrate restriction[55-58]. Increase in whole-body Fatox after treatment is of relevance in this patient population because in a recent cross-sectional study[5] we showed that whole-body Fatox is reduced in patients with NAFLD compared to healthy controls, and that this alteration was associated with the degree of steatosis.

This study comprehensively investigated the independent effects of ER and EX, the cornerstones of lifestyle treatment, on fat and carbohydrate oxidation assessed in different physiological conditions including basal, insulin stimulation, and exercise. This forms an ideal framework to study changes in whole-body energy homeostasis and elucidate mechanisms of change in response to a therapy. Assessment of severity of liver disease, insulin resistance and body composition were conducted using gold standard techniques. A further strength was that the EX program was supervised by an exercise physiologist and was the longest exercise training intervention performed in NAFLD to date.

The randomized controlled trial was powered for detecting within group changes in primary outcome measures (hepatic steatosis and M-value), meaning that type 2 error for other outcome measures cannot be excluded. However, this did not interfere with the interpretation of key results of the present manuscript (*i.e.,* β-hydroxybutyrate concentrations and Fatox during exercise) given that statistically significant differences were still observed. The sample size was relatively small but it was comparable to those from similar studies conducted in NAFLD to date[9,59]. Further, a very specific population was studied: patients were non-diabetic with histologically proven NAFLD and a large proportion (> 75%) of those patients had NASH, which represents an important distinction because patients with NASH are more likely to progress to end stage liver disease[60]. Finally, it must be acknowledged that β-hydroxybutyrate concentrations, while being a commonly used marker of hepatic Fatox[41], do not represent a direct measure of hepatic Fatox. Future studies assessing the effect of lifestyle intervention in NAFLD on rates of hepatic fatty acid uptake, oxidation, and storage using a newly validated method combining 11C-palmitate imaging by positron emission tomography with compartmental modelling[61], would be of interest. Studies including assessment of redox metabolism and gene expression are also warranted.

Based on the length of intervention and type of exercise training provided, the findings of this study suggest that exercise training should not be proposed as a sole therapy for NAFLD. Guidelines should remain unchanged to recommend a combination of both ER and EX given that these interventions provide complementary benefits. EX is particularly beneficial for improving skeletal muscle fat metabolism and CRF, while ER provided greater benefits on hepatic fat metabolism[6]. Future research is required to investigate the impact of different doses and types of exercise programs on the severity of disease as well as on hepatic and whole-body substrate metabolism. Dose and type of exercise are likely to be crucial factors impacting on the clinical benefits of an exercise intervention[62,63]. To date, the beneficial effects of exercise training on NAFLD have been mostly seen in response to aerobic training[59,64-69] or with an aerobic component[9]. It is possible that aerobic exercise training has a greater impact on hepatic steatosis and hepatic Fatox than other training regimes because during aerobic exercise substrate availability is more closely matched with substrate oxidation and energy deficit is greater than during other training regimes.

In conclusion, this study showed ER and EX, standard care interventions for NAFLD management, have specific and complementary benefits on fat metabolism. ER induced weight loss, increased β-hydroxybutyrate concentrations in basal condition, reduced severity of steatosis and severity of disease, but did not lead to changes in substrate oxidation rates during an acute exercise session. EX without weight loss, lead to improvements in substrate oxidation under basal, insulin-stimulated and exercise conditions. However, this dose of circuit exercise training was not sufficient for improvements in β-hydroxybutyrate and severity of liver disease. Increased hepatic Fatox in response to ER could be one of the mechanisms through which the ER group achieved reduction in steatosis.

**ACKNOWLEDGEMENTS**

The authors would like to acknowledge the contribution of clinical and laboratory staff including Julianne Wilson, Sue Cruikshank, Fiona Henderson, Stephane Choquette, William Petchey and Stephanie Ipavec-Levasseur.

**COMMENTS**

***Background***

Lifestyle interventions consisting of diet or diet in conjunction with exercise training are currently the most commonly advocated therapies for non-alcoholic fatty liver disease (NAFLD) management. Limited research has assessed the effect of a lifestyle intervention in NAFLD on whole-body and hepatic fat oxidation in NAFLD.

***Research frontiers***

Understanding the independent effect of diet and exercise on whole-body and hepatic fat oxidation in patients with NAFLD can contribute to elucidate how these interventions impact on the disease and could lead to more specific guidelines for NAFLD management. Exercise training as a treatment option to reduce the burden of NAFLD is an emerging field of research.

***Innovations and breakthroughs***

This study showed diet and exercise, standard care interventions for NAFLD management, have specific and complementary benefits on fat metabolism. Dietary energy restriction provided greater hepatic benefits, while exercise training provided greater peripheral (whole-body) improvements.

***Applications***

Based on the length of intervention and type of exercise program provided (6 mo of circuit exercise training), the findings of this study suggest that exercise training should not be proposed as a sole therapy for NAFLD. Guidelines should continue to recommend a combination of both diet and exercise given that these interventions provide complementary benefits.

***Terminology***

β-hydroxybutyrate is a ketone body produced uniquely by the liver, therefore plasma concentrations of β-hydroxybutyrate are used as an index of hepatic fat oxidation or hepatic ketogenesis.

***Peer-review***

Authors comment adequately the only problem of this study, which is the short number of individuals who completed the study. Results are interesting and the study is well conducted.

**REFERENCES**

1 **Corrado RL**, Torres DM, Harrison SA. Review of treatment options for nonalcoholic fatty liver disease. *Med Clin North Am* 2014; **98**: 55-72 [PMID: 24266914 DOI: 10.1016/j.mcna.2013.09.001]

2 **Marchesini G**, Bugianesi E, Forlani G, Cerrelli F, Lenzi M, Manini R, Natale S, Vanni E, Villanova N, Melchionda N, Rizzetto M. Nonalcoholic fatty liver, steatohepatitis, and the metabolic syndrome. *Hepatology* 2003; **37**: 917-923 [PMID: 12668987 DOI: 10.1053/jhep.2003.50161]

3 **Daly AK**, Ballestri S, Carulli L, Loria P, Day CP. Genetic determinants of susceptibility and severity in nonalcoholic fatty liver disease. *Expert Rev Gastroenterol Hepatol* 2011; **5**: 253-263 [PMID: 21476920 DOI: 10.1586/egh.11.18]

4 **Fabbrini E**, Sullivan S, Klein S. Obesity and nonalcoholic fatty liver disease: biochemical, metabolic, and clinical implications. *Hepatology* 2010; **51**: 679-689 [PMID: 20041406 DOI: 10.1002/hep.23280]

5 **Croci I**, Byrne NM, Choquette S, Hills AP, Chachay VS, Clouston AD, O'Moore-Sullivan TM, Macdonald GA, Prins JB, Hickman IJ. Whole-body substrate metabolism is associated with disease severity in patients with non-alcoholic fatty liver disease. *Gut* 2013; **62**: 1625-1633 [PMID: 23077135 DOI: 10.1136/gutjnl-2012-302789]

6 **Chalasani N**, Younossi Z, Lavine JE, Diehl AM, Brunt EM, Cusi K, Charlton M, Sanyal AJ. The diagnosis and management of non-alcoholic fatty liver disease: Practice guideline by the American Association for the Study of Liver Diseases, American College of Gastroenterology, and the American Gastroenterological Association. *Am J Gastroenterol* 2012; **107**: 811-826 [PMID: 22641309 DOI: 10.1038/ajg.2012.128]

7 **European Association for the Study of the Liver**. EASL-EASD-EASO Clinical Practice Guidelines for the management of non-alcoholic fatty liver disease. *J Hepatol* 2016; **64**: 1388-1402 [PMID: 27062661 DOI: 10.1016/j.jhep.2015.11.004]

8 **Abenavoli L**, Milic N, Peta V, Alfieri F, De Lorenzo A, Bellentani S. Alimentary regimen in non-alcoholic fatty liver disease: Mediterranean diet. *World J Gastroenterol* 2014; **20**: 16831-16840 [PMID: 25492997 DOI: 10.3748/wjg.v20.i45.16831]

9 **Hallsworth K**, Fattakhova G, Hollingsworth KG, Thoma C, Moore S, Taylor R, Day CP, Trenell MI. Resistance exercise reduces liver fat and its mediators in non-alcoholic fatty liver disease independent of weight loss. *Gut* 2011; **60**: 1278-1283 [PMID: 21708823 DOI: 10.1136/gut.2011.242073]

10 **Browning JD**, Baker JA, Rogers T, Davis J, Satapati S, Burgess SC. Short-term weight loss and hepatic triglyceride reduction: evidence of a metabolic advantage with dietary carbohydrate restriction. *Am J Clin Nutr* 2011; **93**: 1048-1052 [PMID: 21367948 DOI: 10.3945/ajcn.110.007674]

11 **Reid BN**, Ables GP, Otlivanchik OA, Schoiswohl G, Zechner R, Blaner WS, Goldberg IJ, Schwabe RF, Chua SC, Huang LS. Hepatic overexpression of hormone-sensitive lipase and adipose triglyceride lipase promotes fatty acid oxidation, stimulates direct release of free fatty acids, and ameliorates steatosis. *J Biol Chem* 2008; **283**: 13087-13099 [PMID: 18337240 DOI: 10.1074/jbc.M800533200]

12 **Savage DB**, Choi CS, Samuel VT, Liu ZX, Zhang D, Wang A, Zhang XM, Cline GW, Yu XX, Geisler JG, Bhanot S, Monia BP, Shulman GI. Reversal of diet-induced hepatic steatosis and hepatic insulin resistance by antisense oligonucleotide inhibitors of acetyl-CoA carboxylases 1 and 2. *J Clin Invest* 2006; **116**: 817-824 [PMID: 16485039 DOI: 10.1172/JCI27300]

13 **Lira FS**, Carnevali LC, Zanchi NE, Santos RV, Lavoie JM, Seelaender M. Exercise intensity modulation of hepatic lipid metabolism. *J Nutr Metab* 2012; **2012**: 809576 [PMID: 22545209 DOI: 10.1155/2012/809576]

14 **Church TS**, Kuk JL, Ross R, Priest EL, Biltoft E, Blair SN. Association of cardiorespiratory fitness, body mass index, and waist circumference to nonalcoholic fatty liver disease. *Gastroenterology* 2006; **130**: 2023-2030 [PMID: 16762625 DOI: 10.1053/j.gastro.2006.03.019]

15 **Nguyen-Duy TB**, Nichaman MZ, Church TS, Blair SN, Ross R. Visceral fat and liver fat are independent predictors of metabolic risk factors in men. *Am J Physiol Endocrinol Metab* 2003; **284**: E1065-E1071 [PMID: 12554597 DOI: 10.1152/ajpendo.00442.2002]

16 **Ross R**, Dagnone D, Jones PJ, Smith H, Paddags A, Hudson R, Janssen I. Reduction in obesity and related comorbid conditions after diet-induced weight loss or exercise-induced weight loss in men. A randomized, controlled trial. *Ann Intern Med* 2000; **133**: 92-103 [PMID: 10896648]

17 **Perseghin G**, Lattuada G, De Cobelli F, Ragogna F, Ntali G, Esposito A, Belloni E, Canu T, Terruzzi I, Scifo P, Del Maschio A, Luzi L. Habitual physical activity is associated with intrahepatic fat content in humans. *Diabetes Care* 2007; **30**: 683-688 [PMID: 17327341 DOI: 10.2337/dc06-2032]

18 **Yasari S**, Prud'homme D, Tesson F, Jankowski M, Gutkowska J, Levy E, Lavoie JM. Effects of exercise training on molecular markers of lipogenesis and lipid partitioning in fructose-induced liver fat accumulation. *J Nutr Metab* 2012; **2012**: 181687 [PMID: 21860785 DOI: 10.1155/2012/181687]

19 **Magkos F**, Lavoie JM, Kantartzis K, Gastaldelli A. Diet and exercise in the treatment of Fatty liver. *J Nutr Metab* 2012; **2012**: 257671 [PMID: 21941637 DOI: 10.1155/2012/257671]

20 **Hickman IJ**, Byrne NM, Croci I, Chachay VS, Clouston AD, Hills AP, Bugianesi E, Whitehead JP, AmaliaGastaldelli, Sullivan TMOM, Prins JB, Macdonald GA. A Pilot Randomised Study of the Metabolic and Histological Effects of Exercise in Non-alcoholic Steatohepatitis. *J Diabetes Metab* 2013 [DOI: 10.4172/2155-6156.1000300]

21 **Byrne N**, Hills A, Meerkin J, Kennedy D. The site specific effectiveness of circuit-weight training on body composition. Proceedings of the 9th Annual Conference of the European College of Sports Science, Clermont-Ferrand, 2004 3-6 July

22 **Kleiner DE**, Brunt EM, Van Natta M, Behling C, Contos MJ, Cummings OW, Ferrell LD, Liu YC, Torbenson MS, Unalp-Arida A, Yeh M, McCullough AJ, Sanyal AJ. Design and validation of a histological scoring system for nonalcoholic fatty liver disease. *Hepatology* 2005; **41**: 1313-1321 [PMID: 15915461 DOI: 10.1002/hep.20701]

23 **Brunt EM**. Nonalcoholic steatohepatitis: definition and pathology. *Semin Liver Dis* 2001; **21**: 3-16 [PMID: 11296695]

24 **Brunt EM**, Kleiner DE, Wilson LA, Belt P, Neuschwander-Tetri BA. Nonalcoholic fatty liver disease (NAFLD) activity score and the histopathologic diagnosis in NAFLD: distinct clinicopathologic meanings. *Hepatology* 2011; **53**: 810-820 [PMID: 21319198 DOI: 10.1002/hep.24127]

25 **Després JP**, Ross R, Boka G, Alméras N, Lemieux I. Effect of rimonabant on the high-triglyceride/ low-HDL-cholesterol dyslipidemia, intraabdominal adiposity, and liver fat: the ADAGIO-Lipids trial. *Arterioscler Thromb Vasc Biol* 2009; **29**: 416-423 [PMID: 19112166 DOI: 10.1161/ATVBAHA.108.176362]

26 **DeFronzo RA**, Tobin JD, Andres R. Glucose clamp technique: a method for quantifying insulin secretion and resistance. *Am J Physiol* 1979; **237**: E214-E223 [PMID: 382871]

27 **Nosadini R**, Avogaro A, Trevisan R, Duner E, Marescotti C, Iori E, Cobelli C, Toffolo G. Acetoacetate and 3-hydroxybutyrate kinetics in obese and insulin-dependent diabetic humans. *Am J Physiol* 1985; **248**: R611-R620 [PMID: 3922234]

28 **Weiss M**, Keller U, Stauffacher W. Effect of epinephrine and somatostatin-induced insulin deficiency on ketone body kinetics and lipolysis in man. *Diabetes* 1984; **33**: 738-744 [PMID: 6146545 DOI: [10.2337/diabetes.33.8.738](http://dx.doi.org/10.2337/diabetes.33.8.738%22%20%5Ct%20%22_blank)]

29 **Havel RJ**, Kane JP, Balasse EO, Segel N, Basso LV. Splanchnic metabolism of free fatty acids and production of triglycerides of very low density lipoproteins in normotriglyceridemic and hypertriglyceridemic humans. *J Clin Invest* 1970; **49**: 2017-2035 [PMID: 5475985 DOI: 10.1172/JCI106422]

30 **Robinson AM**, Williamson DH. Physiological roles of ketone bodies as substrates and signals in mammalian tissues. *Physiol Rev* 1980; **60**: 143-187 [PMID: 6986618]

31 **Frayn KN**. Calculation of substrate oxidation rates in vivo from gaseous exchange. *J Appl Physiol Respir Environ Exerc Physiol* 1983; **55**: 628-634 [PMID: 6618956]

32 **Achten J**, Gleeson M, Jeukendrup AE. Determination of the exercise intensity that elicits maximal fat oxidation. *Med Sci Sports Exerc* 2002; **34**: 92-97 [PMID: 11782653 DOI: [10.1097/00005768-200201000-00015](http://dx.doi.org/10.1097/00005768-200201000-00015%22%20%5Ct%20%22_blank)]

33 **Achten J**, Jeukendrup AE. Maximal fat oxidation during exercise in trained men. *Int J Sports Med* 2003; **24**: 603-608 [PMID: 14598198 DOI: [10.1055/s-2003-43265](http://dx.doi.org/10.1055/s-2003-43265%22%20%5Ct%20%22_blank)]

34 **Achten J**, Jeukendrup AE. Relation between plasma lactate concentration and fat oxidation rates over a wide range of exercise intensities. *Int J Sports Med* 2004; **25**: 32-37 [PMID: 14750010 DOI: [10.1055/s-2003-45231](http://dx.doi.org/10.1055/s-2003-45231%22%20%5Ct%20%22_blank)]

35 **Brandou F**, Dumortier M, Garandeau P, Mercier J, Brun JF. Effects of a two-month rehabilitation program on substrate utilization during exercise in obese adolescents. *Diabetes Metab* 2003; **29**: 20-27 [PMID: 12629444]

36 **Tolfrey K**, Jeukendrup AE, Batterham AM. Group- and individual-level coincidence of the 'Fatmax' and lactate accumulation in adolescents. *Eur J Appl Physiol* 2010; **109**: 1145-1153 [PMID: 20376480 DOI: 10.1007/s00421-010-1453-3]

37 **Aucouturier J**, Rance M, Meyer M, Isacco L, Thivel D, Fellmann N, Duclos M, Duché P. Determination of the maximal fat oxidation point in obese children and adolescents: validity of methods to assess maximal aerobic power. *Eur J Appl Physiol* 2009; **105**: 325-331 [PMID: 19002708 DOI: 10.1007/s00421-008-0907-3]

38 **Kang J**, Rashti SL, Tranchina CP, Ratamess NA, Faigenbaum AD, Hoffman JR. Effect of preceding resistance exercise on metabolism during subsequent aerobic session. *Eur J Appl Physiol* 2009; **107**: 43-50 [PMID: 19504118 DOI: 10.1007/s00421-009-1100-z]

39 **Croci I**, Borrani F, Byrne NM, Wood RE, Hickman IJ, Chenevière X, Malatesta D. Reproducibility of Fatmax and fat oxidation rates during exercise in recreationally trained males. *PLoS One* 2014; **9**: e97930 [PMID: 24886715 DOI: 10.1371/journal.pone.0097930]

40 **Ipavec-Levasseur S**, Croci I, Choquette S, Byrne NM, Cowin G, O'Moore-Sullivan TM, Prins JB, Hickman IJ. Effect of 1-h moderate-intensity aerobic exercise on intramyocellular lipids in obese men before and after a lifestyle intervention. *Appl Physiol Nutr Metab* 2015; **40**: 1262-1268 [PMID: 26575100 DOI: 10.1139/apnm-2015-0258]

41 **Hodson L**, Frayn KN. Hepatic fatty acid partitioning. *Curr Opin Lipidol* 2011; **22**: 216-224 [PMID: 21494141 DOI: 10.1097/MOL.0b013e3283462e16]

42 **Rector RS**, Thyfault JP, Morris RT, Laye MJ, Borengasser SJ, Booth FW, Ibdah JA. Daily exercise increases hepatic fatty acid oxidation and prevents steatosis in Otsuka Long-Evans Tokushima Fatty rats. *Am J Physiol Gastrointest Liver Physiol* 2008; **294**: G619-G626 [PMID: 18174272 DOI: 10.1152/ajpgi.00428.2007]

43 **Rector RS**, Thyfault JP, Laye MJ, Morris RT, Borengasser SJ, Uptergrove GM, Chakravarthy MV, Booth FW, Ibdah JA. Cessation of daily exercise dramatically alters precursors of hepatic steatosis in Otsuka Long-Evans Tokushima Fatty (OLETF) rats. *J Physiol* 2008; **586**: 4241-4249 [PMID: 18617560 DOI: 10.1113/jphysiol.2008.156745]

44 **Holloszy JO**, Coyle EF. Adaptations of skeletal muscle to endurance exercise and their metabolic consequences. *J Appl Physiol Respir Environ Exerc Physiol* 1984; **56**: 831-838 [PMID: 6373687]

45 **Holloway GP**, Bezaire V, Heigenhauser GJ, Tandon NN, Glatz JF, Luiken JJ, Bonen A, Spriet LL. Mitochondrial long chain fatty acid oxidation, fatty acid translocase/CD36 content and carnitine palmitoyltransferase I activity in human skeletal muscle during aerobic exercise. *J Physiol* 2006; **571**: 201-210 [PMID: 16357012 DOI: [10.1113/jphysiol.2005.102178](http://dx.doi.org/10.1113/jphysiol.2005.102178%22%20%5Ct%20%22_blank)]

46 **Schenk S**, Horowitz JF. Coimmunoprecipitation of FAT/CD36 and CPT I in skeletal muscle increases proportionally with fat oxidation after endurance exercise training. *Am J Physiol Endocrinol Metab* 2006; **291**: E254-E260 [PMID: 16670153 DOI: [10.1152/ajpendo.00051.2006](http://dx.doi.org/10.1152/ajpendo.00051.2006%22%20%5Ct%20%22_blank)]

47 **Molé PA**, Oscai LB, Holloszy JO. Adaptation of muscle to exercise. Increase in levels of palmityl Coa synthetase, carnitine palmityltransferase, and palmityl Coa dehydrogenase, and in the capacity to oxidize fatty acids. *J Clin Invest* 1971; **50**: 2323-2330 [PMID: 5096516 DOI: [10.1172/JCI106730](http://dx.doi.org/10.1172/JCI106730%22%20%5Ct%20%22_blank)]

48 **Simoneau JA**, Veerkamp JH, Turcotte LP, Kelley DE. Markers of capacity to utilize fatty acids in human skeletal muscle: relation to insulin resistance and obesity and effects of weight loss. *FASEB J* 1999; **13**: 2051-2060 [PMID: 10544188]

49 **Venables MC**, Jeukendrup AE. Endurance training and obesity: effect on substrate metabolism and insulin sensitivity. *Med Sci Sports Exerc* 2008; **40**: 495-502 [PMID: 18379212 DOI: 10.1249/MSS.0b013e31815f256f]

50 **Talanian JL**, Galloway SD, Heigenhauser GJ, Bonen A, Spriet LL. Two weeks of high-intensity aerobic interval training increases the capacity for fat oxidation during exercise in women. *J Appl Physiol* (1985) 2007; **102**: 1439-1447 [PMID: 17170203 DOI: 10.1152/japplphysiol.01098.2006]

51 **Goto K**, Ishii N, Sugihara S, Yoshioka T, Takamatsu K. Effects of resistance exercise on lipolysis during subsequent submaximal exercise. *Med Sci Sports Exerc* 2007; **39**: 308-315 [PMID: 17277595 DOI: 10.1249/01.mss.0000246992.33482.cb]

52 **Croci I**, Hickman IJ, Wood RE, Borrani F, Macdonald GA, Byrne NM. Fat oxidation over a range of exercise intensities: fitness versus fatness. *Appl Physiol Nutr Metab* 2014; **39**: 1352-1359 [PMID: 25356842 DOI: 10.1139/apnm-2014-0144]

53 **Goodpaster BH**, Katsiaras A, Kelley DE. Enhanced fat oxidation through physical activity is associated with improvements in insulin sensitivity in obesity. *Diabetes* 2003; **52**: 2191-2197 [PMID: 12941756]

54 **Berggren JR**, Boyle KE, Chapman WH, Houmard JA. Skeletal muscle lipid oxidation and obesity: influence of weight loss and exercise. *Am J Physiol Endocrinol Metab* 2008; **294**: E726-E732 [PMID: 18252891 DOI: 10.1152/ajpendo.00354.2007]

55 **Achten J**, Jeukendrup AE. Optimizing fat oxidation through exercise and diet. *Nutrition* 2004; **20**: 716-727 [PMID: 15212756 DOI: [10.1016/j.nut.2004.04.005](http://dx.doi.org/10.1016/j.nut.2004.04.005%22%20%5Ct%20%22_blank)]

56 **Burke LM**, Hawley JA. Effects of short-term fat adaptation on metabolism and performance of prolonged exercise. *Med Sci Sports Exerc* 2002; **34**: 1492-1498 [PMID: 12218744 DOI: [10.1097/00005768-200209000-00015](http://dx.doi.org/10.1097/00005768-200209000-00015%22%20%5Ct%20%22_blank)]

57 **Helge JW**. Adaptation to a fat-rich diet: effects on endurance performance in humans. *Sports Med* 2000; **30**: 347-357 [PMID: 11103848 DOI: [10.2165/00007256-200030050-00003](http://dx.doi.org/10.2165/00007256-200030050-00003%22%20%5Ct%20%22_blank)]

58 **Helge JW**. Long-term fat diet adaptation effects on performance, training capacity, and fat utilization. *Med Sci Sports Exerc* 2002; **34**: 1499-1504 [PMID: 12218745 DOI: [10.1097/00005768-200209000-00016](http://dx.doi.org/10.1097/00005768-200209000-00016%22%20%5Ct%20%22_blank)]

59 **Sullivan S**, Kirk EP, Mittendorfer B, Patterson BW, Klein S. Randomized trial of exercise effect on intrahepatic triglyceride content and lipid kinetics in nonalcoholic fatty liver disease. *Hepatology* 2012; **55**: 1738-1745 [PMID: 22213436 DOI: 10.1002/hep.25548]

60 **Brunt EM**, Kleiner DE, Wilson LA, Unalp A, Behling CE, Lavine JE, Neuschwander-Tetri BA. Portal chronic inflammation in nonalcoholic fatty liver disease (NAFLD): a histologic marker of advanced NAFLD-Clinicopathologic correlations from the nonalcoholic steatohepatitis clinical research network. *Hepatology* 2009; **49**: 809-820 [PMID: 19142989 DOI: 10.1002/hep.22724]

61 **Iozzo P**, Bucci M, Roivainen A, Någren K, Järvisalo MJ, Kiss J, Guiducci L, Fielding B, Naum AG, Borra R, Virtanen K, Savunen T, Salvadori PA, Ferrannini E, Knuuti J, Nuutila P. Fatty acid metabolism in the liver, measured by positron emission tomography, is increased in obese individuals. *Gastroenterology* 2010; **139**: 846-856, 856.e1-6 [PMID: 20685204 DOI: 10.1053/j.gastro.2010.05.039]

62 **Hansen D**, Dendale P, van Loon LJ, Meeusen R. The impact of training modalities on the clinical benefits of exercise intervention in patients with cardiovascular disease risk or type 2 diabetes mellitus. *Sports Med* 2010; **40**: 921-940 [PMID: 20942509 DOI: 10.2165/11535930-000000000-00000]

63 **Ordonez R**, Carbajo-Pescador S, Mauriz JL, Gonzalez-Gallego J. Understanding nutritional interventions and physical exercise in non-alcoholic fatty liver disease. *Curr Mol Med* 2015; **15**: 3-26 [PMID: 25601465 DOI: [10.2174/1566524015666150114110551](http://dx.doi.org/10.2174/1566524015666150114110551%22%20%5Ct%20%22_blank)]

64 **Johnson NA**, George J. Fitness versus fatness: moving beyond weight loss in nonalcoholic fatty liver disease. *Hepatology* 2010; **52**: 370-381 [PMID: 20578153 DOI: 10.1002/hep.23711]

65 **Johnson NA**, Keating SE, George J. Exercise and the liver: implications for therapy in fatty liver disorders. *Semin Liver Dis* 2012; **32**: 65-79 [PMID: 22418889 DOI: 10.1055/s-0032-1306427]

66 **Johnson NA**, Sachinwalla T, Walton DW, Smith K, Armstrong A, Thompson MW, George J. Aerobic exercise training reduces hepatic and visceral lipids in obese individuals without weight loss. *Hepatology* 2009; **50**: 1105-1112 [PMID: 19637289 DOI: 10.1002/hep.23129]

67 **Johnson NA**, van Overbeek D, Chapman PG, Thompson MW, Sachinwalla T, George J. Effect of prolonged exercise and pre-exercise dietary manipulation on hepatic triglycerides in trained men. *Eur J Appl Physiol* 2012; **112**: 1817-1825 [PMID: 21915700 DOI: 10.1007/s00421-011-2158-y]

68 **Keating SE**, Hackett DA, George J, Johnson NA. Exercise and non-alcoholic fatty liver disease: a systematic review and meta-analysis. *J Hepatol* 2012; **57**: 157-166 [PMID: 22414768 DOI: 10.1016/j.jhep.2012.02.023]

69 **Keating SE**, Hackett DA, Parker HM, O'Connor HT, Gerofi JA, Sainsbury A, Baker MK, Chuter VH, Caterson ID, George J, Johnson NA. Effect of aerobic exercise training dose on liver fat and visceral adiposity. *J Hepatol* 2015; **63**: 174-182 [PMID: 25863524 DOI: 10.1016/j.jhep.2015.02.022]

**P-Reviewer:** Abenavoli L, Amodio P, Gonzalez-Reimers E, Li GL, Montalto G, Schlegel A **S-Editor:** Ji FF **L-Editor: E-Editor:**

**Table 1 Characteristics of the study groups at baseline (pre-intervention) and after 6 mo of energy restriction or exercise training (post-intervention)**

|  |  |  |
| --- | --- | --- |
|  | **Energy restriction (*n* = 6)** | **Exercise training (*n* = 10)** |
|  |  **Pre** |  **Post** |  **Pre** |  **Post** |
| Age (yr) | 45.5 ± 13.5 |  |  | 51.8 ± 6.7 |
| Gender (M:F) | 3:3 |  |  | 7:3 |
| BMI (kg/m2) | 33.5 ± 9.0 | 30.0 ± 7.0a | 31.2 ± 3.2 | 30.8 ± 3.5 |
| Fat-mass (%) | 38 ± 9 | 35 ± 11b | 36 ± 7 | 33 ± 6a |
| Fat-free mass (kg) | 54.1 ± 12.3 | 51.3 ± 11.8 | 63.1 ± 14.3 | 64.4 ± 14.2a |
| Waist (cm) | 106 ± 16 | 90 ± 13 | 110 ± 14 | 105 ± 13a |
| Systolic blood pressure (mmHg) | 126 ± 13 | 118 ± 13a | 139 ± 19 | 137 ± 18 |
| Subcutaneous adipose tissue (cm2) | 358 ± 282 | 268 ± 202b | 322 ± 116 | 298 ± 117a |
| Visceral adipose tissue (cm2) | 202 ± 110 | 203 ± 56b | 182 ± 67 | 117 ± 36a |
| Diastolic blood pressure (mmHg) | 83 ± 8 | 75 ± 12 | 88 ± 11 | 83 ± 10 |
| Triglycerides (mmol/L) | 1.6 ± 0.8 | 1.1 ± 0.4 | 2.0 ± 1.3 | 2.0 ± 0.2 |
| HDL cholesterol (mmol/L) | 0.9 ± 0.2 | 1.0 ± 0.3 | 1.0 ± 0.2 | 1.1 ± 0.2a |
| LDL cholesterol (mmol/L) | 3.5 ± 0.8 | 3.0 ± 0.6 | 3.2 ± 1.1 | 3.1 ± 1.0 |
| VLDL cholesterol (mmol/L) | 0.7 ± 0.3 | 0.5 ± 0.2b | 0.9 ± 0.6 | 0.7 ± 0.5 |
| Free fatty acids (mmol/L) | 0.59 ± 0.15 | 0.63 ± 0.23 | 0.59 ± 0.17 | 0.62 ± 0.25 |
|  Glucose (mmol/L) | 5.2 ± 0.3 | 5.0 ± 0.7 | 5.5 ± 0.5 | 5.3 ± 0.4 |
| Insulin (mU/L) | 18 ± 18 | 10 ± 5 | 24 ± 23 | 12 ± 10 |
| M-value (mg/kgFFM per minute) | 4.2 ± 1.4 | 5.2 ± 1.5 | 4.0 ± 0.9 | 5.2 ± 1.6a |
| hs-CPR (mg/L) | 4.9 ± 3.7 | 2.0 ± 1.6 | 3.9 ± 3.6 | 1.5 ± 1.3 |
| Alanine aminotransferase (U/L) | 80 ± 65 | 55 ± 55 | 54 ± 19 | 49 ± 28 |
| Aspartate aminotransferase (U/L) | 40 ± 22 | 28 ± 16 | 38 ± 11 | 39 ± 22 |

Complete-case analysis performed. a*P* < 0.05, within group difference in reponse to the intervention; b*P* value < 0.10, within group trend in reponse to the intervention. Pre-intervention there was no difference between energy restriction and exercise groups in any of the parameters presented (*P* > 0.05). BMI: Body mass index; HDL: High density lipoprotein; LDL: Low density lipoprotein; VLDL: Very low density lipoprotein; hsCRP: High sensitivity C reactive protein.

**Table 2 Resting substrate metabolism pre-intervention and after 6 mo of energy restriction or exercise training (post-intervention) in patients with non-alcoholic fatty liver disease**

|  |  |  |
| --- | --- | --- |
|  | **Energy restriction (*n* = 6)** | **Exercise (*n* = 10)** |
|  | **Pre** | **Post** | ***P*** | **Pre** | **Post** | ***P*** |
| Respiratory Quotient | 0.82 ± 0.04  | 0.80 ± 0.04 | 0.38 |  0.84 ± 0.06  | 0.81 ± 0.06 | **0.05** |
| Fatox (mg/kgFFM per minute) | 1.18 ± 0.25 | 1.46 ± 0.33 | 0.17 | 1.15 ± 0.54 | 1.35 ± 0.48 | 0.08 |
| CHOox (mg/kgFFMV) | 2.33 ± 0.69 | 1.72 ± 0.83 | 0.19 | 2.70 ± 1.24 | 1.90 ± 1.17 | **0.02** |

Complete-case analysis performed. Fatox: Fat oxidation rates; CHOox: Carbohydrate oxidation rates; FFM: Fat-free mass.

**Table 3 Change in substrate metabolism from basal (resting and fasting) to insulin-stimulation conditions pre-intervention and after 6 mo of energy restriction or exercise training (post-intervention)**

|  |  |  |
| --- | --- | --- |
|  | **Energy restriction (*n* = 6)** | **Exercise (*n* = 10)** |
|  | **Pre** | **Post** | ***P*** | **Pre** | **Post** | ***P*** |
| Δ Respiratory quotient  | 0.05 ± 0.05  | 0.08 ± 0.05 | 0.58 |  0.04 ± 0.02  | 0.07 ± 0.05 | 0.11 |
| Δ Fatox (mg/kgFFM per minute) | -0.29 ± 0.46 | -0.56 ± 0.32 | 0.31 | -0.24 ± 0.36 | -0.55 ± 0.35 | 0.06 |
| Δ CHOox (mg/kgFFM per minute) | 0.92 ± 0.98 | 1.41 ± 0.98 | 0.46 | 0.54 ± 0.85 | 1.02 ± 0.93 | 0.18 |

Complete-case analysis performed. Fatox: Fat oxidation rates; CHOox: Carbohydrate oxidation rates; FFM: Fat-free mass; Δ: Change from basal to insulin-stimulated condition.

**Table 4 Maximal aerobic power and substrate oxidation during exercise pre-intervention, and after 6 mo of energy restriction or exercise treatment (post-intervention)**

|  |  |  |
| --- | --- | --- |
|  | **Energy restriction (*n* = 6)** | **Exercise (*n* = 10)** |
|  | **Pre** | **Post** | ***P*** | **Pre** | **Post** | ***P*** |
| VO2peak (mL/kg per minute) | 20.4 ± 5.1 | 20.7 ± 6.4 | 0.73 | 23.9 ± 6.4 | 28.3 ± 6.3 | **< 0.001** |
| VO2peak (mL/kgFFM per minute) | 32.5 ± 5.0 | 31.0 ± 5.4 | 0.31 | 39.2 ± 8.4 | 43.6 ± 7.4 | **0.004** |
| Workload at VO2peak (W) | 121 ± 53 | 121 ± 57  | 0.94 | 176 ± 78 | 224 ± 81 | **< 0.001** |
| MFO (g/min)  | 0.14 ± 0.13 | 0.06 ± 0.04 | 0.17 | 0.17 ± 0.09  | 0.29 ± 0.14 | **0.03** |
| MFO (mg/kgFFM per minute) | 2.5 ± 1.7 | 1.2 ± 0.7 | 0.18 | 2.8 ± 1.5 | 4.4 ± 1.9 | **0.04** |
| Workload at MFO (W) | 44.8 ± 16.5  | 41.3 ± 13.4 | 0.43 | 44.7 ± 19.5 | 76.3 ± 46.0 | **0.03** |
| Fatmax (%VO2peak) | 48.7 ± 14.7 | 47.9 ± 8.8 | 0.62 | 45.2 ± 12.3 | 47.0 ± 7.2 | 0.94 |

Complete-case analysis performed. VO2peak: Peak oxygen uptake; MFO: Maximal fat oxidation; W: Watts; Fatmax: Exercise intensity eliciting maximal fat oxidation; FFM: Fat-free mass.



**Figure 1 Consort diagram describing the flow of patients through the randomised controlled trial.**



**Figure 2 Basal β-hydroxybutyrate concentrations before and after 6 mo of energy restriction (*n* = 6) or exercise training (*n* = 10).** A: Average responses; B: Individual responses. a*P* < 0.05 between pre and post treatment.



**Figure 3 Relationship between change in β-hydroxybutyrate concentrations and relative change in hepatic steatosis in response to 6 mo of energy restriction or exercise training (*n* = 13).** This relationship remained significant after controlling for changes in body weight (*r* = -0.67, *P* = 0.02).

****

**Figure 4 Maximal fat oxidation before and after six months of energy restriction (*n* = 6) or exercise training (*n* = 10); individual data.** a*P* < 0.05 between pre and post intervention.