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ORIGINAL ARTICLE

Clinical Trials Study

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

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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 energy restriction (ER) without changes in structured physical activity (ER; n = 8). Respiratory quotient (RQ) and whole-body fat oxidation rates (Fat_{ox}) were determined by indirect calorimetry under basal, insulin-stimulated and exercise conditions. Severity of disease and steatosis was determined by liver histology; hepatic Fat_{ox} was estimated from plasma β-hydroxybutyrate concentrations; cardiorespiratory fitness was expressed as $\dot{V}O_{2peak}$. 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 mmol/L *vs* 0.12 ± 0.04 mmol/L, P = 0.03) but remained unchanged in response to EX (0.10 ± 0.03 mmol/L *vs* 0.11 ± 0.07 mmol/L, P = 0.39). Basal RQ decreased (P = 0.05) in response to EX, while this change was not significant after ER (P = 0.38). VO_{2peak} (P < 0.001) and maximal Fat_{ox} 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 Fat_{ox} 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

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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.

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INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) is the most prevalent liver disease in industrialised 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 (Fat_{ox}) in resting and fasting conditions, a reduced suppression of Fat_{ox} after insulin stimulation and a lower increase in Fat_{ox} during exercise compared to lean controls[5]. Further, we observed that patients with NAFLD had reduced hepatic Fat_{ox}, 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 Fat_{ox}. Hallsworth *et al*[9] showed that 8 wk of resistance training without weight loss did not change substrate oxidation rates in the basal state (resting and fasting) but increased Fat_{ox} 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 $\dot{V}O_{2peak}$). Therefore, assessment of maximal rate of Fat_{ox} (MFO) and the intensity at which it occurs (Fat_{max}) was not possible, and participants likely were assessed at a lower relative intensity post-intervention (due to improved $\dot{V}O_{2peak}$). 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 Fat_{ox} is also unclear. In response to dietary energy restriction (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 Fat_{ox}), and this was correlated with reduction in steatosis[10]. This is in agreement with findings in animal models showing that an increase in hepatic Fat_{ox} 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 Fat_{ox} and hepatic Fat_{ox} 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 Fat_{ox} and in whole-body substrate oxidation rates under basal, insulin-stimulated and exercise conditions, in patients with NAFLD who completed either 6 mo of dietary energy restriction or circuit exercise training. The second aim was to assess whether changes in CRF, whole-body fat and hepatic Fat_{ox} 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 randomised 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 $\dot{V}O_{2peak}$). 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 Fat_{ox} 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, 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.

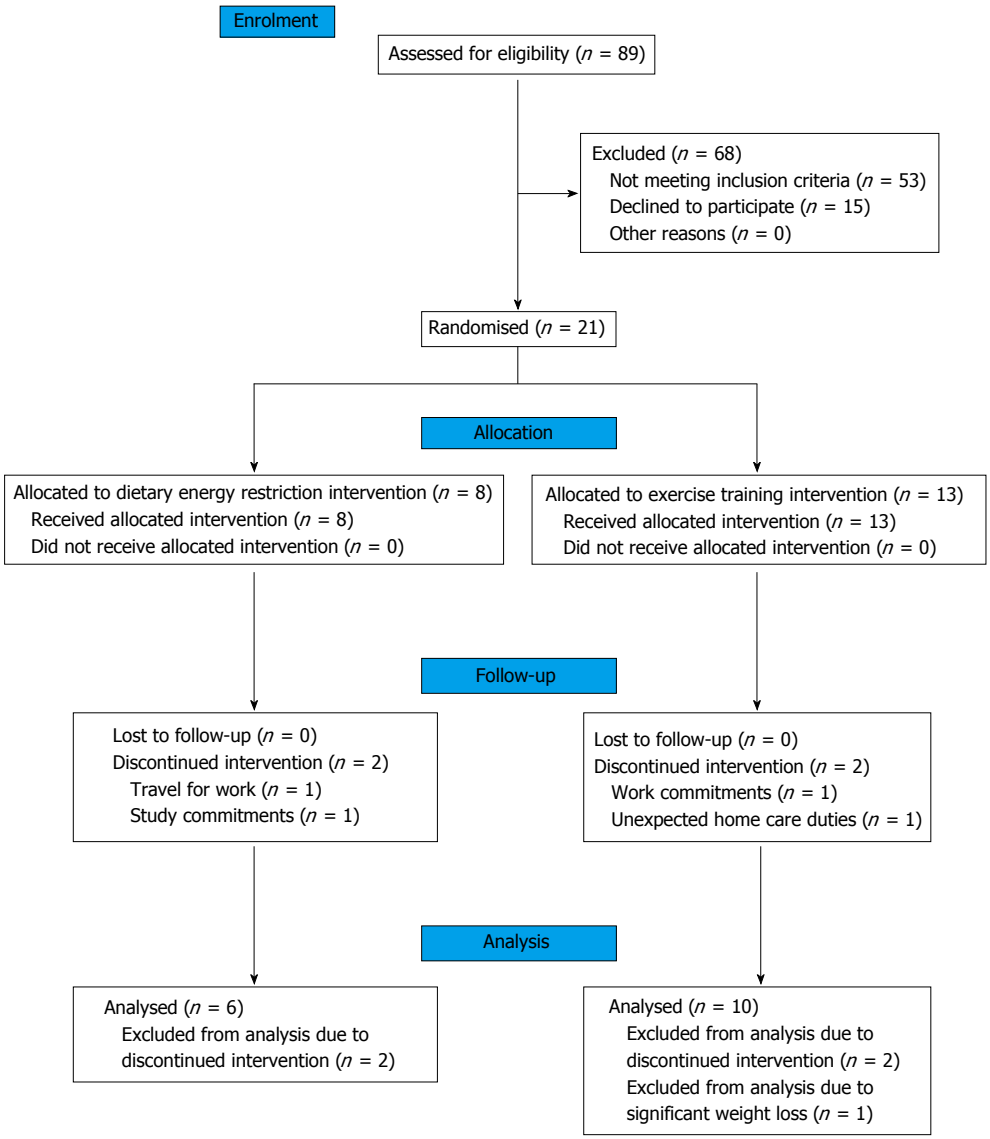

Figure 1 Consort diagram describing the flow of patients through the randomised controlled trial.

Energy restriction

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 Fat_{ox} and CHO_{ox} at multiple intensities (with workload increments occurring every 5 min), and a maximal phase to assess peak oxygen consumption ($\dot{V}O_{2peak}$) (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 Fat_{ox} and CHO_{ox} 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].

Fat_{ox} rates during exercise were estimated from respiratory gazes averaged over the last minute of each exercise stage. Then, the stage at which MFO was achieved was determined, and the corresponding intensity was identified (Fat_{max})[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 ± 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 CHO_{ox} decreased (by 30%, $P = 0.02$), while Fat_{ox} 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

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/m^2)	33.5 ± 9.0	30.0 ± 7.0[a]	31.2 ± 3.2	30.8 ± 3.5
Fat-mass (%)	38 ± 9	35 ± 11[b]	36 ± 7	33 ± 6[a]
Fat-free mass (kg)	54.1 ± 12.3	51.3 ± 11.8	63.1 ± 14.3	64.4 ± 14.2[a]
Waist (cm)	106 ± 16	90 ± 13	110 ± 14	105 ± 13[a]
Systolic blood pressure (mmHg)	126 ± 13	118 ± 13[a]	139 ± 19	137 ± 18
Subcutaneous adipose tissue (cm^2)	358 ± 282	268 ± 202[b]	322 ± 116	298 ± 117[a]
Visceral adipose tissue (cm^2)	202 ± 110	203 ± 56[b]	182 ± 67	117 ± 36[a]
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 ± 0.2[a]
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 ± 0.2[b]	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 ± 1.6[a]
hsCPR (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). M:F: Male: Female; BMI: Body mass index; HDL: High density lipoprotein; LDL: Low density lipoprotein; VLDL: Very low density lipoprotein; hsCRP: High sensitivity C reactive protein.

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 mmol/L *vs* 0.12 ± 0.04 mmol/L, P = 0.03) but remained unchanged in response to EX (0.10 ± 0.03 mmol/L *vs* 0.11 ± 0.07 mmol/L, 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 mmol/L *vs* 0.09 ± 0.03 mmol/L, 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 Fat_{ox} 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

$\dot{V}O_{2peak}$ 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). Fat_{max} 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 (%$\dot{V}O_{2peak}$). Within the EX group, the increase in $\dot{V}O_{2peak}$ (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 $\dot{V}O_{2peak}$ 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

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
Fat_{ox} (mg/kgFFM per minute)	1.18 ± 0.25	1.46 ± 0.33	0.17	1.15 ± 0.54	1.35 ± 0.48	0.08
CHO_{ox} (mg/kgFFM per minute)	2.33 ± 0.69	1.72 ± 0.83	0.19	2.70 ± 1.24	1.90 ± 1.17	0.02

Complete-case analysis performed. Fat_{ox}: Fat oxidation rates; CHO_{ox}: 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 Fat_{ox} (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 CHO_{ox} (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. Fat_{ox}: Fat oxidation rates; CHO_{ox}: 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
$\dot{V}O_{2peak}$ (mL/kg per minute)	20.4 ± 5.1	20.7 ± 6.4	0.73	23.9 ± 6.4	28.3 ± 6.3	< 0.001
$\dot{V}O_{2peak}$ (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 $\dot{V}O_{2peak}$ (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
Fat_{max} (%$\dot{V}O_{2peak}$)	48.7 ± 14.7	47.9 ± 8.8	0.62	45.2 ± 12.3	47.0 ± 7.2	0.94

Complete-case analysis performed. $\dot{V}O_{2peak}$: Peak oxygen uptake; MFO: Maximal fat oxidation; W: Watts; Fat_{max}: Exercise intensity eliciting maximal fat oxidation; FFM: Fat-free mass.

induced weight loss, reduced hepatic steatosis, increased β-hydroxybutyrate concentrations (a marker of hepatic Fat_{ox}) 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 exercise did not lead to improvements in hepatic Fat_{ox} 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 Fat_{ox} 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 Fat_{ox} 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 exercise training on β-hydroxybutyrate concentrations[13]. Results from the present study do not confirm findings from rodent models, which showed that chronic exercise training increased hepatic Fat_{ox}[42] and that the shift from an active to a sedentary lifestyle reduced hepatic Fat_{ox}[43]. Future studies assessing the effects of different training prescriptions (volume, intensity, frequency, duration) and the optimal type of exercise (aerobic *vs* circuit *vs* resistance) 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

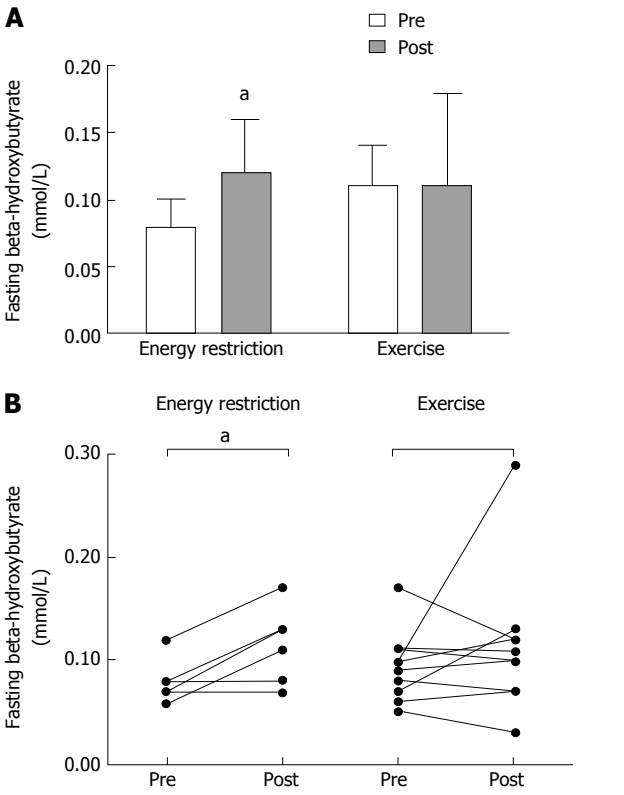

Figure 2 Basal β-hydroxybutyrate concentrations before and after 6 mo of energy restriction (*n* = 6) or exercise training (*n* = 10). A: Average responses; B: Individual responses. [a]*P* < 0.05 between pre and post treatment.

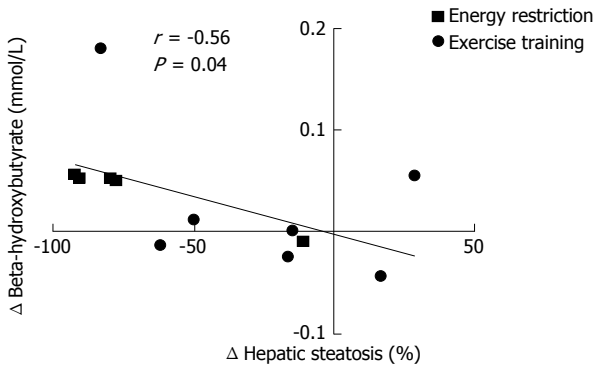

Figure 3 Relationship between change in β-hydroxybutyrate concentrations and relative change in hepatic steatosis in response to 6 mo of energy restriction or exercise training (*n* = 13). This relationship remained significant after controlling for changes in body weight (*r* = -0.67, *P* = 0.02).

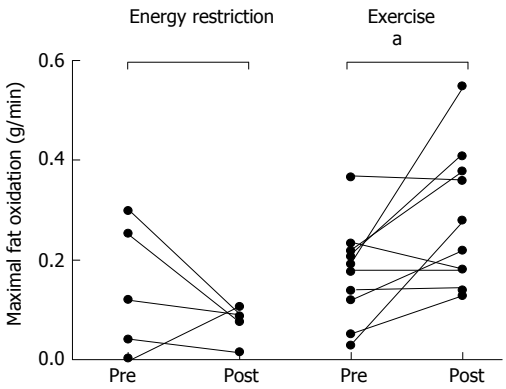

Figure 4 Maximal fat oxidation before and after six months of energy restriction (*n* = 6) or exercise training (*n* = 10); individual data. [a]*P* < 0.05 between pre and post intervention.

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 Fat_{max} 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 Fat_{ox} 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 Fat_{ox} in basal conditions and greater increase in CHO_{ox} in response to insulin stimulation). The increased basal whole-body Fat_{ox} 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 Fat_{ox}, 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 Fat_{ox} after treatment is of relevance in this patient population because in a recent cross-sectional study[5] we showed that whole-body Fat_{ox} 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 randomised 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 Fat_{ox} 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 Fat_{ox}[41], do not represent a direct measure of hepatic Fat_{ox}. 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 ^{11}C-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 exercise training 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 Fat_{ox} 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 Fat_{ox} in response to ER could be one of the mechanisms through which the ER group achieved reduction in steatosis.

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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.

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