Moderate intensity exercise training combined with inulin-propionate ester supplementation increases whole body resting fat oxidation and reduces adiposity in overweight women

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ABSTRACT

Background: Our previous work has shown that oral supplementation with inulin propionate ester (IPE) reduces intra-abdominal fat and prevents weight gain and that oral propionate intake enhances resting fat oxidation. The effects of IPE combined with exercise training on energy substrate utilisation are unknown. The aim of this study was to investigate the impact of 4-weeks IPE supplementation, in combination with a moderate intensity exercise training programme, on whole body fat oxidation and on plasma GLP-1 and PYY.

*Methods***:** Twenty overweight healthy women participated in randomised parallel study and underwent 4 weeks of supervised exercise training either with IPE (EX/IPE group) or Placebo (EX/Placebo group) supplementation. Before and after the intervention participants conducted an experimental trial, which involved collection of expired gas and blood samples in the fasted state and during 7 hours of the postprandial state.

Results: Within groups, the EX/IPE group significantly enhanced the amount of fat (Pre, 24.1 ± 1.2 g; Post, 35.9 ± 4.0 g, $P< 0.05$) oxidised and reduced CHO (Pre,77.8 ± 6.0 g; Post, 57.8 ± 7.7 g, $P< 0.05$) oxidised, reduced body weight (Pre, 77.3 ± 4.2 kg; Post, 76.6 ± 4.1 kg, $P< 0.05$) and body fat mass (Pre, 37.7 ± 1.9 %; Post, 36.9 ± 1.9 %, $P< 0.05$). In EX/Placebo group, changes in amount of fat (Pre, 36.8 ± 3.9 g; Post, 37.0 ± 4.0 g) and CHO (Pre, 62.7 ± 6.5g; Post, 61.5 ±7.4 g) oxidized, body weight (Pre, 84.2 ± 4.3 kg; Post, 83.6 ± 4.3 kg) and body fat mass (Pre, 40.1 ± 1.9 %; Post, 38.7 ± 1.5 %) were not significant. Comparing between groups, change in the amount of fat oxidised was significantly ($P<0.05$) higher for EX/IPE compared with EX/Placebo and there was a trend for difference for amount of CHO oxidised ($P=0.06$) and RER ($P=0.06$). Energy expended was not significantly different ($P>0.05$). The interventions had no impact on fasting or postprandial plasma concentrations of GLP-1 and PYY.

Conclusion: Moderate intensity exercise training programmes when combined with daily oral IPE supplementation may help overweight women to achieve increase in fat oxidation.

The study was registered at clinicaltrials.gov as NCT04016350.

Key words: Exercise, inulin propionate ester, fat oxidation, gut hormones, body weight

1. Introduction

Increasing exercise is important in obesity reduction strategies, inducing negative energy balance driven by increasing energy expenditure [1]. However, the effectiveness of exercise induced weight loss in the absence of caloric restriction remains controversial and is highly individual [2-5]. In healthy overweight women, who participated in 7 week-endurance-type exercise programme and achieved variable changes in fat mass and fat oxidation, energy expenditure and the change in resting fat oxidation were the only statistically significant independent predictors of change in fat mass [6]. This suggests that strategies maximizing resting fat oxidation may enhance body fat mass loss alongside exercise in overweight and obese individuals.

The short chain fatty acid (SCFA) propionate, produced through fermentation of dietary fibre by the gut microbiota, has a range of metabolic benefits [7]. Previous work has reported that that a single dose of oral sodium propionate increased both resting energy expenditure and resting fat oxidation in humans [8]. These findings are in line with a recent report that rectal SCFA infusion, high in propionate, increases resting energy expenditure and fat oxidation in overweight men [9]. Together, these data suggest that increasing propionate production in the gut may be useful in an overall weight management strategy. We developed inulin propionate ester (IPE) to target delivery of propionate to the colon, mimicking high-fibre dietary intake using a modest supplement dose [10].

Thus, the main aim of this study was to investigate the impact of daily IPE supplementation combined with 4-week exercise training programme on whole-body fat oxidation and body fat change in overweight women. Since our previous study [10] reported that acute ingestion of 10 g IPE significantly increased postprandial plasma PYY and GLP-1, and that this effect on the incretin response was lost following long-term (24-week) supplementation, this study also investigated the postprandial anorexigenic gut hormones response.

2. Participants and Methods

This single blinded randomised parallel study was conducted on healthy overweight females with BMI >25 kg/m^2 and 25-45 years of age. Study participants underwent 4 week supervised moderate intensity exercise training combined either with IPE (EX/IPE) or cellulose as placebo (EX/Placebo) supplementation. Before and at the end of the 4-week intervention, participants underwent body weight and body composition measurements, conducted a submaximal exercise test and a 7-hour experimental trial, which involved collection of expired air and blood samples in fasted and postprandial states. Detailed description of the participants, study design and methods are available in the online-only Supplementary Material.

3. Results

3.1. Changes in body weight and body fatness

Physical characteristics of the participants measured before and after 4-week interventions are presented in Supplementary Table 1. Participants of the EX/Placebo group (n=11) exercised at a HR of 146 ± 4 beat·min^{-1}, which corresponded to 61 ± 1 % of the predicted maximal oxygen consumption and the participants of the EX/IPE (n=9) group exercised at a HR of 142 ± 5 beat·min^{-1}, which corresponded to 60 ± 2% of the predicted maximal oxygen consumption. The total energy expenditure of the exercise programmes was not significantly different between groups (EX/Placebo, 5768 ± 412; EX/IPE, 5469 ± 390 kcal) and body weight loss of 0.77 ± 0.16 kg and 0.74 ± 0.17 kg was expected in EX/Placebo and EX/IPE groups, respectively. In the EX/Placebo group, the 4-week exercise programme had no effect (P>0.05) on mean body weight, BMI, body fat mass and body fat percentage whereas in the EX/IPE group post-intervention body weight, BMI, body fat mass and body fat percentage were significantly (P<0.05) lower in comparison to the pre-intervention values The differences

between body weight and body fat changes in EX/Placebo and EX/IPE groups were not significant but in the EX/IPE group responses were less variable.

3.2. Fat and CHO oxidation during 7-hour trials

In EX/Placebo group, four weeks of intervention had no significant impact on fat and CHO oxidation rates while in the EX/IPE group difference in pre- and post-intervention rate of fat oxidation was significant ($P<0.05$, two-way ANOVA, trial effect) (Figure 1). In the EX/Placebo group, the intervention had no impact on total amount of fat and CHO oxidised while in the EX/IPE group intervention increased the amount of fat ($P<0.05$) and reduced the amount of CHO ($P<0.05$) oxidized (Table 1). Comparing between groups, changes in the amount of fat oxidised were significantly ($P<0.05$) different and a trend for difference was observed for amount of CHO oxidised ($P=0.06$) and RER ($P=0.06$). Energy expended was not significantly different ($P>0.05$) (Table 1).

3.3 Appetite-related gut hormones

In both, the EX/Placebo and EX/IPE groups, four weeks of intervention had no significant effect on plasma concentrations of plasma GLP-1 and PYY ($P>0.05$, two-way ANOVA, trial effects). Comparing between groups, changes in time-averaged areas under the responses of PYY or GLP-1 versus time curves were not statistically different (Supplementary Table 2). In EX/IPE group, time averaged areas under the curve of GLP-1 measured during both, pre-intervention and post-intervention trials, were significantly ($P<0.05$, unpaired t-test) higher than in EX/Placebo group while pre-intervention and post-intervention concentrations of PYY were not different between EX/IPE and Ex/Placebo groups (Supplementary Table 2).

4. Discussion

This first-in-human study demonstrates that IPE supplementation leads to increased resting whole-body fat oxidation in the postprandial state when combined with moderate intensity 4-

week exercise programme in overweight women. We found that the change in the amount of fat oxidised during seven hours of the experimental trial was significantly higher in the IPE than Placebo group and the difference in the change in fat oxidations between groups consisted of approximately 10 grams. Thus, the beneficial effects observed on fat oxidation with single dose propionate oral consumption [8] translate into other physiological states, including during exercise training. As in some other studies [11,12] resting fat oxidation was not affected by four weeks exercise training combined with placebo. Thus, enhanced fat oxidation seen in IPE group most likely relates to daily intake of IPE rather than to participation in the exercise programme. We note that fat oxidation measurements were conducted at least 18 hours after intake of the last IPE dose. Thus, supplementation with IPE had a long-term rather than acute effect on resting fat oxidation.

As in majority of exercise training studies without dietary restriction [13], predicted body weight loss was modest and not clinically significant (≤5% weight loss). In the control group intervention had no significant effect on mean body weight and body fatness and as in other studies [4-6, 14] the responsiveness to exercise training was also variable. The IPE group achieved a significant reduction in body weight and body fatness and changes were less variable than in the control group. Taking into consideration that in the control group, changes in fat oxidation were found only in some participants and that in IPE group fat oxidation was enhanced in all participants, our data support the hypothesis that increase in fat oxidation during exercise programmes is important for achieving improvements in body weight and body fat. Further work is required to assess if the effects on body mass and composition over longer duration persist with IPE in well-designed randomised controlled trials.

We also found that IPE supplementation during exercise programme had no impact on plasma concentrations of GLP-1 and PYY. This observation is novel and important and suggests that

GLP-1 and PYY response to IPE is attenuated within 4 weeks and thus persists much shorter than we previously reported in a 24-week intervention study [10]. However, as with fat oxidation measurements, collection of blood for hormone measurements occurred 18-24 hours after intake of the last IPE dose and thus acute elevation GLP-1 and PYY after IPE cannot be ruled out. The finding that concentrations of GLP-1 and PYY were not modified by exercise intervention alone is consistent with findings from other similar studies [5,15].

This study has limitations. Data obtained in this study do not allow to establish causality between IPE induced change in fat oxidation and change in body fatness. This should be investigated by future studies which include measurements of behavioural compensatory variables such as changes in energy intake and energy expenditure of physical activity outside exercise sessions, known to contribute to the responsiveness to exercise training programmes [16-18]. It is possible that increasing the number of participants could lead to significant changes in GLP-1 and PYY concentrations in IPE group and differences between post- and pre-intervention CHO oxidation were seen at additional time points. We note that this was a preliminary study and we had no way to formally calculate a sample size for study outcomes. Thus, with the data from the present study, further appropriately powered, randomised controlled trials to investigate the longer-term effects of IPE on body weight in both men and women alongside prescribed exercise interventions are warranted. Considering appetite regulating hormones beyond GLP-1 and PYY [19] will also be important in future studies.

In conclusion, this study demonstrates that adding IPE to moderate intensity exercise programmes, applied to overweight women, achieves an increase in whole-body resting fat oxidation.

Author contributions

DJM, DM, KG, ESC, CMT, GF contributed to the concept development and designed study; TP, ER recruited participants and conducted the experimental work; ER, DM performed appetite hormone analysis and statistical analyses; DJM, DM drafted the manuscript; All authors contributed to revisions of the manuscript. None of the authors had a personal or financial conflict of interest to disclose.

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Disclosure Summary

GF, DJM and TP are named inventors on the patent WO2014020344A1.

Competing Interests

None of declare.

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Figure Legends

Figure 1. Rate of fat and carbohydrate (CHO) oxidation, and energy expenditure (EE) in the fasted state (0h) and during post-breakfast (0-180min) and post-lunch (180-420 min), measured before (Week 0) and after 4-week exercise intervention (Week 4) combined with Placebo (Ex/Placebo group, n=11) and Inulin Propionate Ester (Ex/IPE group, n = 9) supplementation. Values are means ± SEs.*Significant (P<0.05) difference at corresponding time points.

289 **Table 1.** Total amount of fat and carbohydrate (CHO) oxidised, time averaged respiratory
290 exchange ratio (RER) and energy expended (EE) during 7 hours of the experimental trial
291 conducted before (Week 0) and after (Week 4) the exercise programme conducted with placebo
292 (EX/Placebo) and inulin propionate ester (EX/IPE). All values are mean ± SEM.

	EX/Placebo (n=11)		EX/IPE (n=9)		*P value*
	Week 0	Week 4	Week 0	Week 4	
Fat (g)	31.3 ± 2.4	33.0 ± 2.3	24.1 ± 1.2	35.9 ± 4.0[a]	0.02
CHO (g)	69.3 ± 6.0	64.6 ± 6.9	77.8 ± 5.9	57.8 ± 7.7[a]	0.06
RER	0.85 ± 0.01	0.84 ± 0.01	0.87 ± 0.01	0.84 ± 0.02	0.06
EE (kcal)	551 ± 13	549 ± 14	540 ± 14	531 ± 25	0.64

293 *P* value are for difference between change in Ex/Placebo and EX/IPE groups. [a] Significant
294 ($P<0.05$) difference between Week 4 and Week 0 in corresponding group.

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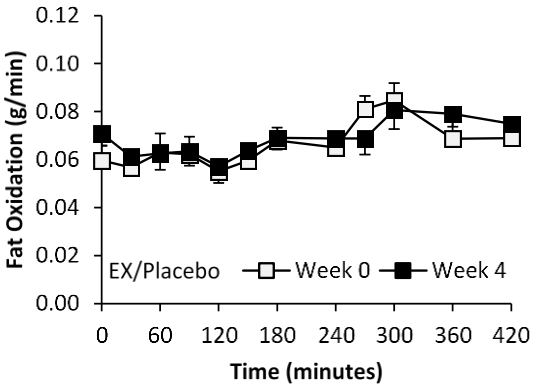
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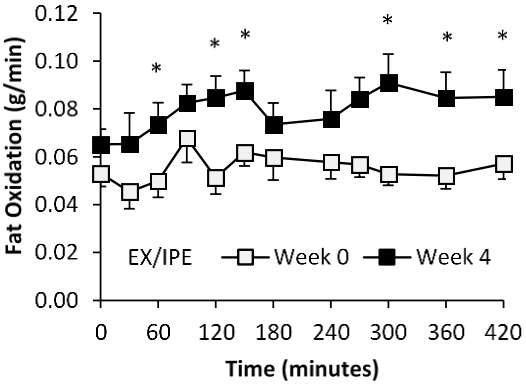
Time (minutes)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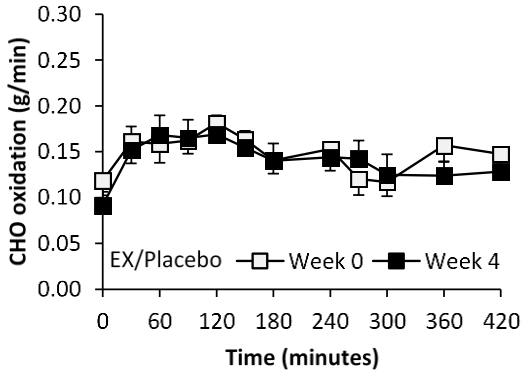
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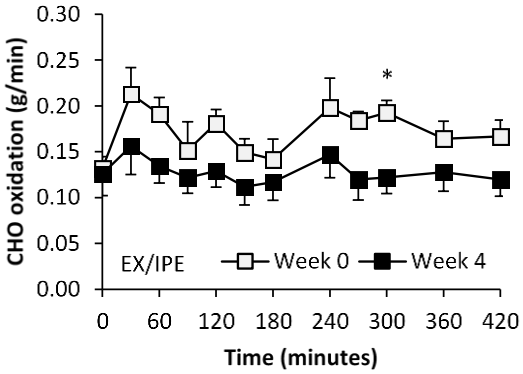
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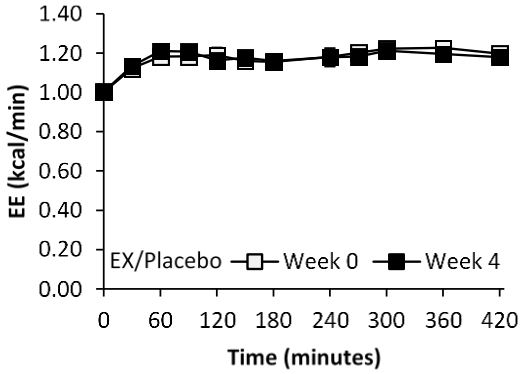
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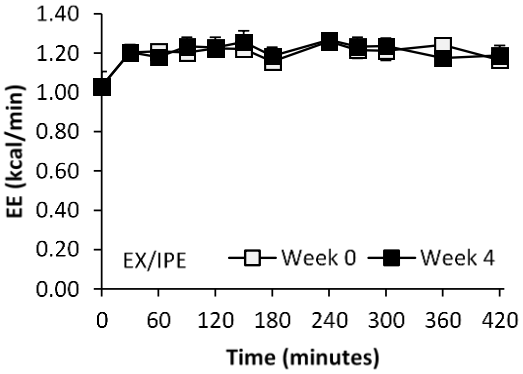
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SUPPLEMENTARY MATERIAL

Participants

Healthy overweight females with BMI >25 kg/m^2 and 25-45 years of age were recruited through advertisements and word of mouth on the campus of the University of Glasgow or from other public places. Participants were required to be sedentary, non-smokers, with stable body weight for two months prior to the study enrolment, not pregnant, free of medication, nutritional supplementation or following any specific diet and with no antibiotic use for the past three months. Participants with chronic illness, eating disorders and history of gastrointestinal operations were excluded. All participants gave written informed consent. The Ethics Committee of the College of Medical, Veterinary and Life Sciences of the University of Glasgow approved the study (Project Number: 200140132). Study started in February 2015 and was completed in June 2017.

Study design overview

This was a single blinded randomised parallel study. Study participants underwent 4 week supervised moderate intensity exercise training combined either with IPE (EX/IPE) or cellulose as placebo (EX/Placebo) supplementation at doses of 10g/day. Random allocation of the participants was achieved by chance procedure and conducted by one of the researchers. After the assignment to the intervention the participants were blinded in relation to the supplement.

Cellulose and IPE were provided as sachets containing 10g of white powder and participants were asked to consume one sachet per day with breakfast. Participants were advised to take supplements with water or any juice which they include habitually in their breakfast. Empty sachets were returned and counted as a measure of compliance. Before the start of the study and at the end of the 4-week intervention, participants were asked to conduct a submaximal exercise test. Prior to the first and after the second submaximal exercise test, participants of both groups conducted a 7-hour experimental trial which involved collection of expired air and blood samples in fasted and postprandial states. Body weight and body composition were measured in the fasted state. Prior to the first 7-hour experimental trial, participants were asked to record their diet for 3 days and replicate this intake prior to the second 7-hour experimental trial. For training sessions all participants were given free access to the University of Glasgow Sports Centre. 7-hour experimental trials and submaximal tests were conducted at the metabolic investigation laboratories of Glasgow University (West Medical and New Lister Buildings)

7-hour experimental trials

On the morning of each experimental trial, participants reported to the metabolic research unit between 8:00 and 9:00 a.m. after an overnight fast. After anthropometric and body composition measurements participants laid supine on the bed with their head resting on pillow and expired gas was collected. Following this, a venous cannula was inserted into an antecubital vein and after 10 minutes a baseline blood sample was collected. Participants were then asked to consume a standardised breakfast and after four hours a standardised lunch. Further expired air samples and blood samples were collected at 1-hour intervals. The test breakfast consisted of butter croissant, chocolate spread, whole milk, double cream, milkshake powder and sugar providing 1 g fat, 1.2 g carbohydrate, 0.25 g protein and 15 kcal energy per kg body mass. The test lunch consisted of white bread, mild cheddar cheese, butter, potato crisps, whole milk, double cream, milkshake powder and sugar providing 0.8 g fat, 1.1 g carbohydrate, 0.35 g protein, 13 kcal energy per kg of body mass. Breakfast and lunch were identical in both experimental trials and were served in a standardized way.

Submaximal exercise test

The test was conducted on a treadmill (Trackmaster Treadmills, Full Vision, Inc., Kansas, USA). After a 4-minute warm-up (walking on treadmill at 3.5 km/h), participants walked on the treadmill at a constant speed of 5 or 5.5 km/hour with the incline being increased by 2% every 4 minutes. The whole test consisted of 4 to 6 stages and therefore lasted from 16 to 24

minutes. The test was terminated once the participant reached 85% of their aged-predicted maximal heart rate (HR_{max} = 220 – age). During the last minute of each 4-minute stage, HR was recorded via a heart rate monitor (Polar Sports Tester, Polar Electro Oy, Kempele, Finland), rate of perceived exertion (RPE) was indicated by the participant on the Borg scale (Borg et al., 2010) and an expired air sample was collected by Douglas bag method. Expired air samples were analysed through a gas analyser (1440 Gas Analyser, Servomex, UK) and maximal oxygen consumption ($\dot{V}O_2$ max) was predicted by extrapolation of the HR against $\dot{V}O_2$ plot to age-predicted maximum HR. Data obtained during submaximal tests were used to predict the intensity at which each participant exercised and the energy expenditure of the

Exercise training sessions

Exercise training consisted of four weekly sessions of endurance type exercise (treadmill, cycle ergometer or cross-trainer). Timing of the training sessions was agreed between investigator and the participant and was based on the participant's availability. The duration of the exercise sessions was 30, 40, 50 and 60 minutes for weeks 1, 2, 3 and 4, respectively. Participants were asked to exercise at an individual pre-determined work rate and achieve 60-65 % of their predicted $\dot{V}O_2$ max with HR being recorded every 5 minutes using heart rate monitors (Polar Sports Tester, Polar Electro Oy, Kempele, Finland). All exercise sessions were supervised by a researcher. Total net energy expenditure of the exercise intervention was determined from heart rate obtained in the exercise sessions and individual relationships of the heart rate versus oxygen uptake obtained during submaximal tests.

Expired gas collection and analyses during 7-hour trials

Expired gas was collected and analysed by computerised open-circuit ventilated hood system (Oxycon Pro, Germany). After volume and gas calibrations of the apparatus, participants were instructed to lay supine and be still and awake during the measurement. Once comfortable, a clear plastic canopy (weight, 550 g; dimensions, 19.6 x 12.99 x 9.44 inc) was placed on the participant's head and expired gas was collected after each blood sample collection for the duration of 20 minutes. Values of oxygen consumption ($\dot{V}O_2$) and carbon dioxide production ($\dot{V}CO_2$) rates were recorded every 30 seconds and averaged prior to calculations of the respiratory exchange ratio (RER) and fat and CHO oxidation, and rate of energy expenditure [1]:

Fat Oxidation (g/min) = ($\dot{V}O_2$ - $\dot{V}CO_2$) / 0.57

CHO Oxidation (g/min) = (1.40 x $\dot{V}CO_2$ - $\dot{V}O_2$) / 0.57

Energy Expenditure (kJ/min) = (CHO oxidation x 15.6) + (fat oxidation x 39)

RER= $\dot{V}CO_2$/ $\dot{V}O_2$

Anthropometric and body composition measurements

Height was measured to the nearest 0.5 cm using a stadiometer (Seca, Leicester, UK). Body mass and body fatness were measured by leg-to-leg bioelectrical impedance scales (TBF-300, TANITA, Cranela, UK). Height was determined using standard protocol.

Blood sample analysis

Venous blood samples were collected in ethylenediamine tetra-acetic acid (EDTA) coated evacuated tubes (Greiner Bio-One, Kremsmünster, Austria). Tubes containing blood samples were immediately placed on ice and then centrifuged at 4°C, 3000 rpm for 15 minutes (Hettich D-78532 Universal 320 R Centrifuge, Tuttiligen, Germany). Plasma was dispensed in 0.5 mL aliquots into labeled sterilized micro-centrifuge cap tubes and kept at –80°C until analysis. For analysis, plasma samples were allowed to thaw and then were centrifuged for a few seconds to ensure plasma is mixed and there is no sediment. Commercial ELISA kits were used to measure concentration of plasma GLP-1 (Merck, Millipore, Bioscience Division, UK) and PYY (Merck, Millipore, Bioscience Division, UK). Coefficients of variation (CVs) were <8% for both, GLP-1 and PYY assays.

Statistical analysis

For non-normally distributed data, statistical analysis was performed following log10 transformation. Data on fat and CHO oxidation, energy expenditure and appetite hormones were analysed by two-way (time and trial effects) repeated measures ANOVA, followed by post hoc Tukey test. Amount of total fat and CHO oxidised and energy expended during seven hours of the trial within group were compared by paired t-test while changes in these measures between groups were compared by independent t-test. For all tests, the significance level was accepted at $P<0.05$. Statistical analysis was performed using Minitab (version 17.3.1; Minitab, Inc. State College, PA) and Statistica (version 10.0; StatSoft, Inc. Tulsa, OK).

References

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Supplementary Table 1. Physical characteristics of the participants before (Week 0) and after (Week 4) the exercise intervention conducted with placebo (EX/Placebo) and inulin propionate ester (EX/IPE). Values are mean ± SEM.

	EX/Placebo		EX/IPE		
	Week 0	Week 4	Week 0	Week 4	*P* value
Age (y)	26.8 ± 0.94	-	29.3 ± 1.54	-	
Weight (kg)	79.4 ± 3.3	79.1 ± 3.4	77.3 ± 4.2	76.6 ± 4.1[a]	0.47
BMI (kg/m^2)	29.0 ± 1.1	28.9 ± 1.2	29.4 ± 2.2	29.0 ± 1.9[a]	0.36
Fat mass (kg)	30.9 ± 2.6	30.7 ± 2.7	29.8 ± 3.2	28.8 ± 3.2[a]	0.45
Body Fat (%)	38.3 ± 1.6	37.2 ± 1.1	37.7 ± 1.9	36.9 ± 1.9[a]	0.51
$\dot{V}O_2$max (ml/kg/min)	32.4 ± 1.2	34.2 ± 1.2[a]	30.0 ± 1.0	33.0 ± 1.9[a]	0.82

BMI, Body Mass Index; $\dot{V}O_2$max, maximal oxygen consumption.

P values are for difference between change in Ex/Placebo and EX/IPE groups.

[a] Significant ($P<0.05$) difference between Week 4 and Week 0 in corresponding group.

Supplementary Table 2. Time-averaged areas under plasma concentrations of YY (PYY) and GLP-1 versus time curves during post-breakfast (0-3 hours), post-lunch (3-7hours) period and the entire period (0-7 hours) of the experimental trial conducted before (Week 0) and after (Week 4) the exercise intervention with placebo (EX/Placebo) and inulin propionate ester (Ex/IPE) and change (Δ) in time averaged area under the curve between week 4 and week 0 in corresponding group. Values are presented as Mean ± SE.

	Week 0	Week 4	Δ	Week 0	Week 4	Δ	*P*
PYY							
0-3 h	101.3 ± 11.9	95.6 ± 9.8	-5.7 ± 7.9	89.8 ± 10.0	102.2 ± 8.6	12.4 ± 8.0	0.7
3-7 h	124.4 ± 12.4	119.3 ± 11.9	-5.1 ± 8.1	110.0 ± 11.0	114.9 ± 12.8	4.9 ± 5.9	0.3
0-7 h	113.8 ± 11.5	109.7 ± 10.8	-4.1 ± 6.6	98.1 ± 10.9	108.4 ± 9.8	10.4 ± 6.9	0.2
GLP-1							
0-3 h	29.4 ± 3.6	28.2 ± 4.3	-1.2 ± 2.1	39.5 ± 6.7[a]	40.3 ± 5.4[b]	0.8 ± 2.8	0.6
3-7 h	27.8 ± 3.5	26.6 ± 4.0	-1.2 ± 1.8	41.0 ± 6.1[a]	45.0 ± 5.0[b]	4.0 ± 3.4	0.4
0-7 h	28.5 ± 3.5	27.2 ± 4.1	-1.3 ± 1.8	40.0 ± 6.3[a]	43.6 ± 5.4[b]	3.5 ± 2.5	0.3

P for difference between change in EX/Placebo and EX/IPE groups. [a] Significantly different ($P<0.05$) from Week 0 values in EX/Placebo group; [b] Significantly different ($P<0.05$) from Week 4 values in EX/Placebo group.