Effect of 1-h moderate intensity aerobic exercise on intramyocellular lipids of obese men before and after a lifestyle intervention

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Abstract

Intramyocellular lipids (IMCL) are depleted in response to an acute bout of exercise in lean endurance-trained individuals, however it is unclear whether changes in IMCL content are also seen in response to acute and chronic exercise in obese individuals. We used magnetic resonance spectroscopy in 18 obese men and 5 normal-weight controls to assess IMCL content before and after an hour of cycling at the intensity corresponding with each participant's maximal whole-body rate of fat oxidation (Fat_{max}). Fat_{max} was determined via indirect calorimetry during a graded exercise test on a cycle ergometer. The same outcome measures were re-assessed in the obese group after a 16-week lifestyle intervention comprising dietary calorie restriction and exercise training. At baseline, IMCL content decreased in response to 1-hour cycling at Fat_{max} in controls (2.8±0.4 to 2.0±0.3 a.u., -39%, p=0.02), but not in obese (5.4±2.1 vs. 5.2±2.2 a.u., p=0.42). The lifestyle intervention lead to weight loss (-10.0±5.4 kg, p<0.001), improvements in VO_2max (+5.2±3.4 mL/kg/min), maximal fat oxidation rate (+0.19±0.22 g/min) and a 29% decrease in homeostasis model assessment score (all p<0.05). However, when the 1-hour cycling at Fat_{max} was repeated after the lifestyle intervention, there remained no observable change in IMCL (4.6±1.8 vs. 4.6±1.9 a.u., p=0.92). In summary, there was no IMCL depletion in response to 1-hour cycling at moderate intensity either before or after the lifestyle intervention in obese men. An effective lifestyle intervention including moderate intensity exercise training did not impact rate of utilisation of IMCL during acute exercise in obese men.

Keywords: Exercise training, obesity, energy metabolism, Magnetic Resonance Spectroscopy, muscle, ectopic fat, maximal fat oxidation, intramyocellular triglycerides, diet.

Introduction

Intramyocellular lipid (IMCL) content is elevated in individuals who are obese or have type 2 diabetes, and is associated with insulin resistance in sedentary subjects (Moro et al. 2009). Endurance-trained athletes, paradoxically, also display an elevated IMCL storage, despite being markedly insulin sensitive (Russell 2004). Studies assessing changes in IMCL content after lifestyle interventions showed that diet-induced reduction in body weight resulted in declined IMCL content in obesity and type 2 diabetes (Anastasiou et al. 2010; Toledo et al. 2008). Exercise training has reported mixed results with a number of studies showing an increase in IMCL (Goodpaster et al. 2001; Meex et al. 2010; Shaw et al. 2012), but others showing a decrease (Louche et al. 2013) or no change (Devries et al. 2013). The effect of an exercise-training program on IMCL appears to vary depending on the population studied: a recent study compared three groups and reported reduction in IMCL content of type 2 diabetic but not of obese or lean participants (Bajpeyi et al. 2012). Little research has been conducted on the effect of a real-life lifestyle intervention comprising exercise training as well as diet induced weight loss on the IMCL content of non-diabetic obese men.

In addition to changes in IMCL pools in response to diets or chronic exercise (exercise training programs), there is growing interest in the effects of an acute bout of endurance exercise on IMCL content. There is evidence supporting the contribution of IMCL to fat oxidation during an acute bout of moderate intensity aerobic exercise (50-75% of peak oxygen consumption) lasting an hour or longer in endurance trained normal weight individuals (Badin et al. 2013; Coen and Goodpaster 2012; Shepherd et al. 2012). Indeed, in trained normal weight individuals changes in IMCL content in response to acute exercise have been detected with different techniques (muscle biopsies, magnetic resonance spectroscopy (MRS), oil staining) and in different muscle groups (vastus lateralis, soleus,

tibialis anterior) (Coen and Goodpaster 2012). However, to date it has not been determined whether an acute bout of moderate intensity exercise leads to a reduction in IMCL content of non-diabetic obese men.

Also, the capacity of obese individuals to increase IMCL utilisation during an acute bout of aerobic exercise, as an adaptive response to chronic training, has never been assessed. The work of Dube et al. (2008) and Haus et al. (2011) provides support for the effectiveness of short-term exercise in mediating insulin sensitivity in conjunction with favourable alterations in lipid partitioning and enhanced skeletal muscle oxidative capacity. However these changes were in the absence of significant weight losses, and the utilisation of IMCL during exercise was not examined. Understanding the effects of acute and chronic exercise on IMCL content of obese individuals can provide insights into the pathophysiology of obesity and can inform training prescription for this population.

The objective of this study was to assess the effect of an acute bout (1-h) of moderate intensity aerobic exercise on IMCL content of obese men before and after 4 months of lifestyle intervention comprising dietary energy restriction and aerobic exercise training. A secondary aim was to assess the effect of this intervention on pre-exercise IMCL stores of obese men.

Materials and methods

Subjects. Eighteen obese (BMI ≥ 30 kg/m^2), non-diabetic adult men (44 ± 7 y) were recruited. Participants had no significant renal, hepatic or cardiovascular disease. None had significant weight loss prior to the trial. Diabetes was excluded by a standard oral glucose tolerance test (OGTT). In addition, five age-matched (44 ± 9 y) healthy-weight (BMI 24.3 ± 2.1 kg/m^2, range 21.4–26.9; percentage body fat <25%) recreationally trained men were recruited as a control group. All participants provided written informed consent, which was approved by the Human Research Ethics Committees at the Princess Alexandra Hospital, Wesley Hospital and The University of Queensland.

Study Design. Baseline measures for obese and normal-weight control participants include body composition, biochemical analysis of blood samples and a graded exercise test on a cycle ergometer. This test was used to assess maximal aerobic power ($\dot{V}O_{2max}$), as well as maximal fat oxidation (MFO) and the exercise intensity at which this occurs (Fat_{max}). The week following these measures, Magnetic Resonance Spectroscopy (MRS) of the soleus muscle was performed to quantify IMCL content before and after 1 hour of cycle ergometer exercise performed at the mechanical workload (in Watts) corresponding to Fat_{max}. Subsequently, obese participants progressed into a 16-week intensive lifestyle intervention with weekly review by a dietician and an exercise physiologist; all outcome measures were reassessed at the end of the lifestyle intervention.

Diet intervention. Individualised energy restrictions were calculated for each participant with a recommended macronutrient composition of diet of 50% carbohydrate, 30% fat and 20% protein (NHMRC 2013).

126 ***Home-based exercise intervention.*** Exercise prescription aimed for 1500 kcal/week through
127 aerobic exercises such as walking or biking at an intensity of 65-85% of maximal heart rate
128 (Hordern et al. 2009). Data from exercise sessions was stored in a Polar M31 heart rate
129 monitor (Polar Electro Oy, Oulu, Finland) and manually downloaded weekly to give an
130 objective measure of intensity, duration and energy expenditure.

131 ***Biochemical analysis.*** Blood samples were collected in the morning after a 10 hour fast.
132 Glucose was analysed with an automated Hitachi Modular D&P analyser (Roche, Australia).
133 Insulin was assayed using an immunoenzymometric assay with fluorescence detection using
134 the Tosoh AIA-600 analyser (South San Francisco, CA, USA). Total cholesterol, high
135 density lipoprotein cholesterol (HDL-C), low density lipoprotein cholesterol (LDL-C) and
136 triglycerides (TG) were measured as previously reported (Wong et al. 2006). Insulin
137 sensitivity was calculated using the homeostatic model of assessment of insulin resistance
138 (HOMA-IR) (Matthews et al. 1985).

139 ***Total Body composition.*** In the obese group, body composition was determined by dual
140 energy x-ray absorptiometry (DXA) and analysed using the DPX-L adult software, version
141 1.33 (DPX-Plus; Lunar Corp, Madison, WI), as previously described (Croci et al. 2013;
142 Hickman et al. 2013). In the control group, fat free mass (FFM) was estimated using the
143 semi-mechanistic model equation of Janmahasatian et al. (2005). This equation was chosen
144 as it has been validated against bioelectric-impedance analysis and dual x-ray absorptiometry.

145 ***Substrate oxidation and maximal aerobic power test.*** Maximal aerobic power and substrate
146 utilization were assessed with a graded exercise test on a cycle ergometer. The test was
147 conducted in a fasted state on a Monark 824E cycle ergometer with pedal straps tightened
148 over toes to maximise soleus muscle involvement. Testing included a sub-maximal phase to

assess fat oxidation at various intensities, and a maximal phase to determine peak oxygen consumption ($\dot{V}O_{2max}$). Pedalling frequency was maintained between 70-75 revolutions per minute throughout the test. The workload was increased by adding a resistance of 0.3 to 0.4 kg at each stage, until the respiratory exchange ratio was above 1.0 during the last minute of the stage. Stages lasted 4 minutes and were separated by 4-minute rest intervals, in which the participants were seated motionless on the cycle ergometer. The maximal phase started at a workload corresponding to one stage below the intensity reached at the end of the submaximal phase, and workload was incremented every minute until volitional exhaustion.

Inspiratory volume and respired gas concentrations were measured using the Moxus Modular System (AEI Technologies, Pittsburgh, PA) and calibrated using standard protocols (Roffey et al. 2007). Whole-body fat oxidation rates were calculated using stoichiometric equations and appropriate energy equivalents, with the assumption that the urinary nitrogen excretion rate was negligible (Frayn 1983). Average values of oxygen consumption and carbon dioxide production were calculated during the last minute of each submaximal exercise stage. Fat oxidation values determined at each stage of the exercise test were graphically depicted as a function of exercise intensity. The stage at which the value of measured fat oxidation was maximal (maximal fat oxidation) was determined, and the corresponding intensity identified (Fat_{max}) (Achten et al. 2002).

Testing session were performed in the morning after a 12 hour overnight fast and in standardised conditions. Participants were asked to refrain from consuming caffeine and alcohol, and to abstain from performing any kind of strenuous exercise during the 24 hours prior to those sessions. Standardisation of pre-test conditions were in line with previous studies (Achten and Jeukendrup 2003; Achten and Jeukendrup 2004; Achten et al. 2002;

172 Aucouturier et al. 2009; Brandou et al. 2003; Croci et al. 2014a; Croci et al. 2014b; Kang et
173 al. 2009; Tolfrey et al. 2010).
174
175 ***Magnetic Resonance Imaging (MRI) and Spectroscopy (MRS).*** Pre-acute exercise
176 measurements were taken after a 10-hour overnight fast. Post-acute exercise measurements
177 were started 3 to 5 min after the completion of the 1-h cycling. Subjects were instructed to
178 refrain from strenuous physical activity for three days prior to testing. Abdominal images
179 were assessed using a Siemens Sonata 1.5T system (Erlangen, Germany) in the supine
180 position using standard array coils during a single breath-hold using fourteen axial FISP
181 images, 8mm thick, centred on L4. When the patients extended beyond the 400mm maximum
182 field-of-view addition images were acquired offset in the left then right direction to ensure
183 full coverage of the abdomen. The average results for the four slices that were best aligned
184 with L4 were reported.

185 Right leg soleus IMCL was assessed by MRS using a 4T Bruker/Siemens MedSpec whole
186 body scanner with a custom-built transmit/receive knee coil. Single voxel spectra were
187 measured using the PRESS technique (Kimmich and Hoepfel 1986) with the following
188 parameters: TR = 2 sec, TE = 30 ms, data points = 2048, bandwidth = 2000 Hz, voxel size =
189 15x15x15 mm, no water suppression, averages =128. Voxels were positioned within the
190 muscle using standard T1 weighted structural images, avoiding obvious large regions of
191 extracellular fat.

192 Depletion of soleus IMCL has been previously demonstrated in lean trained individuals
193 (Brechtel et al. 2001; Larson-Meyer et al. 2002).

194 ***MRI/MRS quantification.*** Images were analysed by a single operator using the polygon ROI
195 tool in SliceOMatic (version 4.3; Tomovision, Montréal, Canada). Quantity of each
196 metabolite was estimated by fitting the spectra using prior knowledge in AMARES from the
197 jMRUI package. IMCL values are presented in arbitrary units as represented by the total area
198 under the curve of IMCL_CH_2 + IMCL_CH_3 divided by the total area under the curve of
199 water (Torriani et al. 2005) and multiplied by 100. The variability of this method was
200 assessed by repeating the measurements 5 times on the same day, with a coefficient of
201 variance of 6.2%.

202 ***Statistical Analysis.*** A priori sample size calculations showed that 20% depletion in IMCL
203 can be detected with 16 participants, with 80% power and alpha 0.05 (De Bock et al. 2007).
204 Statistical analyses were performed using SPSS for Windows version 14.0 (SPSS Inc.
205 Chicago, IL, USA). Variables were summarized as mean □ standard deviation (SD).
206 Variables that were not normally distributed were log transformed prior to analysis. The
207 degree of association between continuous variables was assessed using Pearson's correlation
208 coefficient. Differences in the means of variables between baseline and after intervention and
209 before and after exercise were assessed using paired two- tailed Student t-tests, and baseline
210 comparisons between control and obese participants were conducted using independent two-
211 tailed Student's t-tests. A p value ≤ 0.05 was considered statistically significant.

212

213 **Results**

214

215 The characteristics of obese participants before and after the lifestyle intervention are
216 presented in Table 1. During the intervention, dietary energy intake decreased from 2535 ±
217 827 kcal/d to 1925 ± 655 kcal/d ($p = 0.002$), with no significant change in macronutrient
218 composition (dietary fat 34 ± 4% vs. 31 ± 6%, $p = 0.1$). Subjects achieved an average energy
219 expenditure of 1207 ± 971 kcal per week through exercise (interquartile range: 252-1628
220 kcal/week), which corresponded to 3.0 ± 2.4 h/week of exercise. On average, exercise
221 training was performed at an intensity of 68.4 ± 7.4% of the maximum heart rate.

222 The lifestyle intervention promoted a decrease in body weight (-8.6%, $p < 0.001$) and waist
223 circumference (-12.7 cm, $p < 0.001$). Significant improvements were also observed in total
224 cholesterol ($p = 0.005$), LDL-cholesterol ($p = 0.01$), and insulin resistance as measured by
225 HOMA-IR ($p = 0.03$). A 20% increase in $\dot{V}O_{2max}$ relative to body weight ($p = 0.001$) was
226 observed (Table 2), which remained significant after correcting for FFM. The increase in
227 $\dot{V}O_{2max}$ relative to FFM was 12% ($p < 0.001$).

228

229 The effect of 1-h cycling on intramyocellular lipids of obese men before and after a lifestyle
230 intervention is presented in Table 2 and Figure 1. At baseline, there was no change in IMCL
231 content of obese participants in response to acute exercise (5.4 ± 2.1 to 5.2 ± 2.2, $p = 0.42$).
232 In contrast, after 1-h of identical relative exercise protocol, the control group decreased
233 IMCL content from 2.8 ± 0.4 to 2.0 ± 0.3 (-39%, $p = 0.02$).

234 After lifestyle intervention, the obese group remained unable to reduce IMCL content during
235 1-h cycling at Fat_{max} (4.64 ± 1.76 vs. 4.62 ± 1.94, $p = 0.92$). IMCL stores pre- acute exercise
236 (in resting and fasting conditions) tended to decrease in response to the lifestyle intervention
237 in the obese group (5.4 ± 2.1 vs. 4.6 ± 1.8, $p = 0.09$).

Linear regression analyses revealed no association between pre-intervention IMCL and pre-intervention HOMA-IR ($p = 0.85$) and no association between the pre-post intervention change in HOMA-IR and the pre-post intervention change in IMCL ($p = 0.49$). However, the pre-post intervention change in HOMA-IR was associated with changes in waist circumference ($r = 0.50$, $p = 0.05$) and in maximal lipid oxidation ($r = -0.53$, $p = 0.03$).

Discussion

There is evidence supporting the contribution of IMCL to fat oxidation during an acute bout of exercise in trained normal weight individuals (Badin et al. 2013; Coen and Goodpaster 2012), however it is unclear whether obese men display a similar response. This study showed that obese untrained men do not deplete IMCL of soleus muscle as a substrate source during an hour of moderate-intensity exercise. Four months of chronic aerobic training in conjunction with dietary restriction, which resulted in 9% weight loss, 12% improvement in CRF and 58% increase in whole-body fat oxidation rates, did not alter this inability to deplete IMCL in response to acute aerobic exercise. In contrast, in non-obese trained controls, IMCL content decreased by 39% after an identical relative bout of exercise.

In this study participants exercised at Fat_{max} (~60% $\dot{V}O_{2max}$), which is an exercise intensity similar to that adopted in a number of previous studies that demonstrated IMCL reduction in lean individuals (Shepherd et al. 2012; Watt et al. 2002). The duration of the exercise bout adopted in the present study (1h) has been shown to effectively induce IMCL depletion in lean individuals (Shepherd et al. 2012; White et al. 2003) as well as in the lean cohort assessed in this study. Longer duration of exercise (1.5 up to 2-3h) have been shown to lead to greater IMCL depletion in lean individuals; Watt et al. (2002) suggest that only the first measure of intramuscular lipids after two hours of exercise showed depleted stores. It cannot be excluded that such longer duration might have lead to an observable IMCL depletion in obese individuals, however applying a greater dose of exercise (e.g. 1.5-3 h) may not have been clinically translatable for this population. The choice of 1h of exercise adopted in the present study is in line with the advice from the Institutes of Medicine (IOM 2002) and the International Association for the Study of Obesity (Saris et al. 2003) for the purpose of preventing unhealthy weight gain or regain in obese individuals who have lost weight.

While muscle fibres of obese individuals contain more IMCL, the utilisation of these lipids for energy production appears to be altered. This could be due to a number of factors including skeletal muscle fatty acid oxidation capacity, lower number of mitochondria and IMCL location within muscle cells (Moro et al. 2008). It has been shown that lipids aggregate near the sarcolemma in lean subjects, while in obesity, a higher percentage of lipids aggregate within the central area of muscle fibres (far the from sarcolemma), which might result in an impaired ability of the cell to oxidise lipid from this depot (Malenfant et al. 2001). The lack of depletion of IMCL in response to an acute bout of aerobic exercise observed in obese individuals in this study is in line with results from a recent study that showed no change in IMCL content in 8 healthy untrained overweight men (BMI 29 kg/m^2) after 1.5h of cycling at 50% of $\dot{V}O_{2\max}$ (Nellemann et al. 2014). It must also be noted that the obese group, due to lower CRF, performed at the acute bout of exercise at a lower absolute exercise intensity (in Watts) compared to the trained control group. Egger et al. (2013) recently studied a group of lean moderately trained participants and showed that IMCL depletion in response to acute exercise was not correlated with $\dot{V}O_{2\max}$ nor with the absolute exercise intensity at which the acute bout of exercise was performed, however it cannot be excluded that the absolute exercise intensity need to be above a certain threshold for IMCL depletion to occur.

Another novel aspect of this study is that this was the first study to assess whether lifestyle intervention can lead to greater IMCL depletion in response to acute exercise in obese individuals. The observation that obese individuals did not deplete IMCL during acute exercise even after 4 months of lifestyle intervention could be due to the fact that: i) the dose of exercise training completed was highly variable between subjects (interquartile range: 252-

293 1628 kcal/week), and ii) the post-intervention CRF of this obese cohort, despite improved,
294 was still lower than what is commonly seen in untrained lean individuals (ACSM 2006). It is
295 possible that a minimal CRF is required to observe detectable changes in IMCL in response
296 to an acute bout of aerobic exercise or that genetics may play a greater role in the regulation
297 of IMCL mobilisation than does training *per se*.
298
299 Maximal whole-body fat oxidation was markedly increased in response to the intervention in
300 obese men but this was not accompanied by IMCL depletion during an acute bout of aerobic
301 exercise. This suggests that the increase in whole-body fat oxidation during exercise was
302 mostly accounted for by the increased plasma free fatty acid oxidation during exercise and
303 not by the oxidation of local stores such as IMCL. During prolonged endurance exercise,
304 adipose tissue lipolysis supplies free fatty acid to the working muscle, and plasma free fatty
305 acid availability regulates intramuscular use of lipids (van Loon et al. 2005). This suggests
306 that elevated plasma free fatty acid levels may inhibit the mobilisation and/or oxidation rate
307 of intramuscular stores. In addition to the increased contribution from plasma free fatty acid,
308 it cannot be excluded that post-intervention, there also has been an increased contribution
309 from IMCL from other muscles groups. Future studies using stable isotope methodologies to
310 determine the source of lipids utilised during acute exercise in obesity are warranted.
311
312 In response to the diet and exercise training lifestyle intervention, IMCL stores of obese
313 individuals assessed in resting and fasting conditions (before undergoing the acute bout of
314 exercise) tended to decrease. These findings are in line with studies which showed a
315 decreased IMCL in response to diet induced weight loss (Anastasiou et al. 2010; Toledo et al.
316 2008), or a recent study showing that 8 weeks of endurance exercise training without weight

317 loss lead to a 42% reduction in IMCL content in 10 obese male (Louche et al. 2013). On the
318 other hand, increase or no change in IMCL were seen in response to some exercise training
319 programs (Devries et al. 2013; Meex et al. 2010; Shaw et al. 2012) or interventions including
320 both exercise and diet modification (Haus et al. 2011; He et al. 2004). The discrepancies
321 observed between studies could be due to the type and volume of exercise undertaken, the
322 amount of weight loss achieved (Goodpaster et al. 2000) or the different metabolic status of
323 the populations studied (Bajpeyi et al. 2012). Indeed, given that the relationship between
324 IMCL content and insulin action is "U shaped", for certain individuals a metabolic
325 improvement (a move along the curve to the right) is represented by a decrease IMCL
326 content, while for others it is represented by an increase IMCL content. Future studies should
327 consider the effect of negative energy balance on mobilisation of different fat depots during
328 exercise when the energy deficit of the diet and exercise are matched as was done by Ross et
329 al. (2000). Findings from this study add clinically relevant and translatable information to the
330 literature which had previously focused mostly on the separate effects of diet and exercise
331 training interventions on IMCL.
332
333 The target group of this study was non-diabetic obese men (60% of whom were severely
334 obese). A comprehensive assessment of metabolic profile and body composition was
335 performed. The results of this study have good external validity as it reflects the effect of a
336 real-life, achievable lifestyle intervention. A 4T magnet was used for the MRS analyses,
337 which enabled accurate distinction between IMCL and extramyocellular lipid in skeletal
338 muscle. The study has statistical power to detect changes in IMCL content (Torriani et al.
339 2005), therefore lack of changes observed is not attributable to sample size. Sample size in
340 the present study was similar or greater compared to previous studies assessing the effect of

341 acute and chronic exercise on IMCL content by MRS (Bucher et al. 2014; De Bock et al.
342 2007; Egger et al. 2013).

343 Methodological aspects deserve some discussion and include the muscle group assessed, the
344 exercise modality adopted and the timing of IMCL assessment. Firstly, although analysis of
345 the quadriceps and gluteal muscles, as opposed to the soleus, would appear ideal because of
346 their involvement during cycling, it was not logistically possible, as the quadriceps of obese
347 individuals did not fit inside the coil. Mobilisation of soleus muscle IMCL during exercise
348 has been demonstrated by others (Brechtel et al. 2001; Larson-Meyer et al. 2002) and
349 contains a high proportion of type 1 muscle fibres (Polgar et al. 1973), and three to four times
350 the IMCL content of the tibialis anterior (van Loon and Goodpaster 2006). Importantly, the
351 control group of this study demonstrated that measurable differences in soleus IMCL could
352 be demonstrated in response to an identical protocol of 1-h cycling at Fat_{max}. Secondly,
353 testing exercise capacity and thresholds using cycle ergometry is recognised to be the
354 preferred modality when measuring physical power and adaptations to exercise, particularly
355 in clinical populations, as external mechanical work is able to be easily quantified, the
356 stepwise increases in work rate are more effectively controlled. Moreover, within limits, the
357 mechanical efficiency is independent of bodyweight (Astrand et al. 2003; Cooper and Storer
358 2001). Given the expected weight-loss as a consequence of the lifestyle intervention, using a
359 walking protocol to analyse changes in energy utilisation during exercise would be
360 confounded by changes in external work due to reduced weight bearing. Thirdly, there is
361 evidence that timing of IMCL measurement after exercise is an important experimental
362 consideration given that elevated plasma free fatty acids following exercise may replenish
363 IMTG stores (Coen and Goodpaster 2012). To avoid such confounder, in the present study
364 the delay between acute exercise and IMCL assessment was minimal (3-5 minutes). Finally,

365 it must be noted that IMCL content uniquely reflects the total IMCL content at the time of the
366 assessment (i.e MRS or biopsy) and does not inform on the amount of oxidation and
367 synthesis that has occurred. Therefore, it cannot be excluded that in the obese group a rapid
368 rate of IMCL re-synthesis partly explained the lack of detectable change in IMCL contents
369 (Nellemann et al. 2014).
370
371 In conclusion, we have shown that obese untrained non-diabetic men do not deplete IMCL of
372 soleus muscle in response to an hour of moderate-intensity exercise, and that this was not
373 changed by a lifestyle intervention, despite the decrease of 9% body weight and an
374 improvement in CRF, whole-body fat oxidation and insulin sensitivity.
375

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557 **Tables**

558

559 **Table 1.** Characteristics of the obese participants at baseline and after 4 months of lifestyle
560 intervention

	Obese Baseline n=18	Obese Post Intervention n=18	P Value
Body weight (kg)	115.9 ± 12.9	105.9 ± 12.7	<0.001
Waist circumference (cm)	120.5 ± 10	107.8 ± 10.5	<0.001
FFM (kg)	70.1 ± 8	68.1 ± 7	0.063
FM (kg)	41.8 ± 7.3	33.7 ± 8.5	<0.001
FM (%)	37.2 ± 3.9	32.7 ± 5.7	<0.001
Abdominal subcutaneous fat (cm^2)	464 ± 94	401 ± 117	<0.001
Abdominal visceral fat (cm^2)	204 ± 110	141 ± 79	<0.001
Glucose (mmol/L)	5.51 ± 0.62	5.07 ± 0.49	0.003
Insulin (mU/L)	17 ± 11	13 ± 7	0.037
HOMA-IR	4.19 ± 2.91	2.99 ± 1.48	0.031
Total-C (mmol/L)	5.37 ± 1.08	4.89 ± 1	0.005
HDL-C (mmol/L)	1.32 ± 0.27	1.36 ± 0.32	0.386
LDL-C (mmol/L)	3.16 ± 0.91	2.86 ± 0.83	0.013
TG (mmol/L)	1.99 ± 0.84	1.47 ± 0.76	0.073

561
562 BMI, Body mass index; FFM, Fat free mass; FM, Fat mass; HOMA-IR, Homeostatic model
563 assessment of insulin resistance; Total-C, total cholesterol; HDL-C, high density lipoprotein;
564 LDL-C, low density lipoprotein; TG, triglycerides. Values are means ± SD. P values are the
565 change with intervention using a paired t-test.

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569 **Table 2.** Effects of 4 months lifestyle intervention on cardiorespiratory fitness, fat oxidation
570 during acute exercise and intramyocellular lipids of obese men

	Lean control n=5	**Obese Baseline n=18**	**Obese Post intervention n=18**
Age (y)	44 ± 9	44 ± 7	–
BMI (kg/m^2)	24.3 ± 2.1	36.8 ± 3.6*	33.6 ± 3.6*‡
$\dot{V}O_{2max}$ (ml/min)	5174 ± 724	2939 ± 590*	3222 ± 732*‡
$\dot{V}O_{2max}$ (ml/kg/min)	57.9 ± 2.7	25.6 ± 5.7*	30.8 ± 8*‡
$\dot{V}O_{2max}$ (ml/kgFFM/min)	74.4 ± 4.5	42.0 ± 8.2*	47.3 ± 9.1*‡
Maximal fat oxidation (g/min)	0.68 ± 0.08	0.33 ± 0.19*	0.52 ± 0.28‡
Fat_{max} intensity			
% $\dot{V}O_{2max}$	59.8 ± 8.4	61.9 ± 12.4	61.6 ± 12.5
$\dot{V}O_2$ (ml/kg/min)	35.6 ± 3.8	15.4 ± 2.8*	18.5 ± 4.6*‡
Absolute (Watts)	210 ± 41	90 ± 24*	104 ± 32*
RER	0.85 ± 0.03	0.89 ± 0.06	0.84 ± 0.07‡
IMCL *pre- acute exercise* (A.U.)	2.81 ± 0.42	5.39 ± 2.08*	4.64 ± 1.76*
IMCL *post- acute exercise* (A.U.)	2.02 ± 0.34†	5.29 ± 2*	4.62 ± 1.94*

571
572 BMI, Body Mass Index; IMCL, intramyocellular lipids; A.U., arbitrary units; $\dot{V}O_{2max}$,
573 Maximal aerobic power; Fat_{max}, exercise intensity at which fat oxidation is maximal; FFM,
574 Fat free mass; RER, respiratory exchange ratio.
575 (*) $p \leq 0.05$ compared to control group, (†) $p \leq 0.05$ compared to pre-exercise within group, (‡)
576 $p \leq 0.05$ compared to pre-intervention.
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579

580 **Figures**

581 **Figure 1.** Effect of 1-h cycling on intramyocellular lipids of obese men before and after a
582 lifestyle intervention. Values are means ± SD. A.U., arbitrary units. IMCL content
583 decreased in response to an acute bout of exercise (1-hour cycling at Fat_{max}) in normal
584 weight controls (p=0.02), but not in obese participants before (p=0.42) or after
585 (p=0.92) the lifestyle intervention. The lifestyle intervention tended to decrease pre-
586 exercise IMCL content in obese men (p=0.09).

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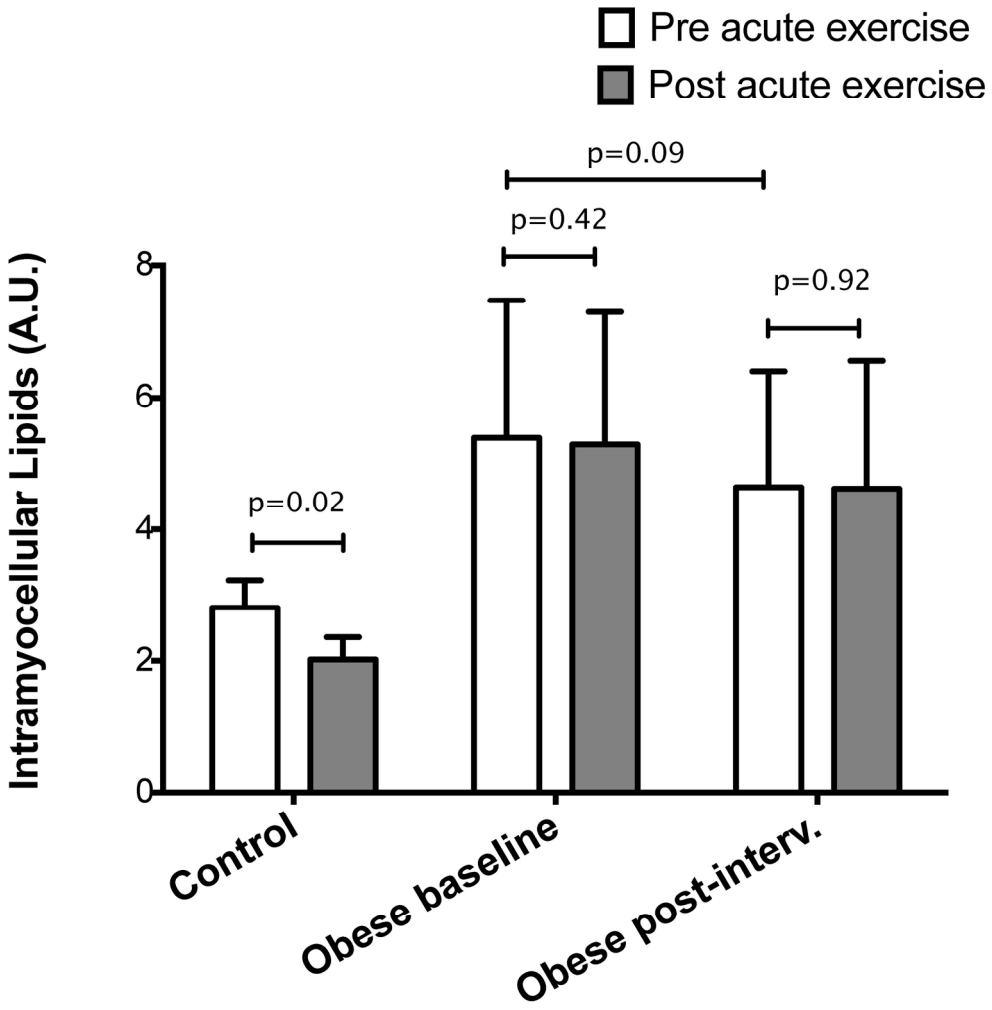

Effect of 1-h cycling on intramyocellular lipids of obese men before and after a lifestyle intervention. Values are means ± SD. A.U., arbitrary units. IMCL content decreased in response to an acute bout of exercise (1-hour cycling at Fatmax) in normal weight controls (p=0.02), but not in obese participants before (p=0.42) or after (p=0.92) the lifestyle intervention. The lifestyle intervention tended to decrease pre-exercise IMCL content in obese men (p=0.09).
182x185mm (300 x 300 DPI)