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Obesity-induced decreases in muscle performance are not reversed by weight loss

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Running title: Effect of weight loss on muscle

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Conflict of Interest

The authors declare no conflict of interest.

27 **Abstract**

28 **Background/Objectives:** Obesity can affect muscle phenotypes, and may thereby constrain
29 movement and energy expenditure. Weight loss is a common and intuitive intervention for
30 obesity, but it is not known whether the effects of obesity on muscle function are reversible
31 by weight loss. Here we tested whether obesity-induced changes in muscle metabolic and
32 contractile phenotypes are reversible by weight loss.

33 **Subjects/Methods:** We used zebrafish (*Danio rerio*) in a factorial design to compare energy
34 metabolism, locomotor capacity, muscle isometric force and work-loop power output, and
35 myosin heavy chain composition between lean fish, diet-induced obese fish, and fish that
36 were obese and then returned to lean body mass following diet restriction.

37 **Results:** Obesity increased resting metabolic rates ($p < 0.001$) and decreased maximal
38 metabolic rates ($p = 0.030$), but these changes were reversible by weight-loss, and were not
39 associated with changes in muscle citrate synthase activity. In contrast, obesity-induced
40 decreases in locomotor performance ($p = 0.0034$), and isolated muscle isometric stress ($p =$
41 0.01), work loop power output ($p < 0.001$), and relaxation rates ($p = 0.012$) were not reversed
42 by weight loss. Similarly, obesity-induced decreases in concentrations of fast and slow
43 myosin heavy chains, and a shift towards fast myosin heavy chains were not reversed by
44 weight loss.

45 **Conclusion:** Obesity-induced changes in locomotor performance and muscle contractile
46 function were not reversible by weight loss. These results show that weight loss alone may
47 not be a sufficient intervention.

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52 **Introduction**

53 Skeletal muscle is essential for locomotion and posture ¹, as well as for whole-body
54 energy homeostasis and metabolism ^{2,3}. Hence, any impairment of muscle function will
55 impact on the health and fitness of the whole organism. Obesity has detrimental effects on
56 metabolic phenotypes and muscle function ⁴. The consequences of these effects will be
57 compounded if they persist in individuals that have undergone successful weight-loss
58 therapy. It is therefore essential to determine the reversibility of obesity-induced
59 physiological changes in order to predict the potential consequences of obesity and the
60 efficacy of treatments. Our aim was to determine whether diet-induced obesity impairs
61 locomotor capacity and muscle function, and whether any effects are reversible with weight
62 loss. Skeletal muscle function is dependent on the contractile and calcium signaling proteins
63 that mediate contraction and relaxation, and on energy metabolism to supply the necessary
64 ATP ⁵. We therefore investigated metabolism in parallel with muscle contractile function.

65 Many signalling pathways associated with energy homeostasis are conserved among
66 vertebrates and are similar in a number of model species, including humans, rodents, and
67 zebrafish ^{6,7}. One of the principal mediators of obesity-induced metabolic dysfunction is a
68 reduction in concentration and activity of the sirtuin SIRT1^{4,8}. SIRT1 is a NAD⁺-dependent
69 histone deacetylase that promotes expression of a range of metabolic regulators such as PGC-
70 1alpha ⁹, and thereby regulates mitochondrial function ¹⁰. Mice lacking SIRT1 had reduced
71 activity and reduced rates of oxygen consumption, leading to an overall decrease in energy
72 expenditure ¹¹. Conversely, increased expression of SIRT1 led to increased energy
73 expenditure¹². Obesity-induced mitochondrial dysfunction in skeletal muscle manifests as
74 decreased TCA cycle (citrate synthase) activity and electron transport chain flux ¹³. It would
75 be expected therefore that this reduction in maximal metabolic capacities would lead to a

76 reduction in metabolic scope, which represents the energy available for activity and
77 locomotion.

78 Locomotor performance is determined by dynamic muscular contractility rather than
79 by isometric force production, and the capacity of muscles to produce work during the
80 shortening and lengthening cycle, and the passive resistance to stretch determine muscle
81 power output (work-loop performance) ^{14,15}. When normalized to muscle mass, work-loop
82 power output of isolated skeletal muscle was reduced in faster muscle fibre types of obese
83 mice ¹⁶. A possible cause for changes in muscle power output are obesity-induced shifts in
84 the expression of slow (oxidative) type I myosin heavy chains ¹⁷⁻¹⁹, although fibre type shifts
85 can differ between males and females ¹⁹. Obesity also altered locomotor capacity ²⁰ and the
86 metabolic cost of locomotion ²¹, which could be associated with changes in muscle
87 contractile properties. Although obesity is well known to constrain physical performance^{22,23},
88 beyond a single mouse study ²⁴ the effect of obesity on muscle power output are unknown,
89 and it remains to be shown whether there is a link between muscle power output and
90 locomotion, and whether any obesity-induced changes are reversible with weight loss.

91 Weight loss reversed obesity-induced increases in pro-inflammatory proteins ²⁵,
92 reductions in adiponectin levels²⁶, impaired lymphatic function ²⁷, metabolic dysfunction
93 ^{28,29}, and reduction in slow type I myosin heavy chains ¹⁷. However, at least with respect to
94 kidney function it is not clear whether the effects of obesity are reversible by weight loss ³⁰.
95 Overall, it may be expected that obesity-induced declines in physiological function are
96 reversed by weight loss. However, the physiological effects of obesity are so complex that it
97 is difficult to extrapolate between physiological systems. Hence, we tested whether obesity-
98 induced impacts on muscle and locomotor function are reversible by weight loss.

99 We used zebrafish to test the hypotheses that a) obesity reduces metabolic scope and
100 muscle citrate synthase activity because of mitochondrial dysfunction; b) obesity reduces

101 isolated muscle power output; we predicted that these changes in contractile function are
102 associated with decreases in whole-animal locomotor performance, and changes in myosin
103 heavy chain composition; c) obesity-induced changes in metabolism, skeletal muscle and
104 locomotor phenotypes are reversible by weight loss.

105

106 **Materials and Methods**

107 *Study animals and treatments*

108 All procedures were performed with the approval of the University of Sydney Animal
109 Ethics Committee (approval #723). Adult zebrafish (*Danio rerio*) were obtained from a
110 commercial supplier (Livefish, Bundaberg, Australia) and maintained in plastic tanks (600 x
111 450 x 250 mm; 1-2 fish l⁻¹) with dechlorinated water at 25°C, and a 12h dark:12 h light
112 photoperiod for two weeks before experimentation, and fed with commercial fish flakes
113 (Wardley's, The Hartz Mountain Company, Secaucus, USA; 46% protein, 6% fat). After two
114 weeks fish were randomly allocated to one of three groups: 1) control fish were fed once a
115 day to satiety for 9-10 weeks; 2) obese fish fed three times per day to satiety for 9-10 weeks;
116 3) obese-lean fish were fed three times per day for 4-5 weeks, then once per day for 4-5
117 weeks. We took photos of each fish (with an Exilim camera, Casio, Japan) to determine
118 standard length (in ImageJ software, NIH, USA), and we weighed fish before treatments,
119 again at the time when obese-lean fish were switched to the lean diet, and at the end of the
120 treatments immediately before measurements were taken.

121

122 *Metabolism and swimming performance*

123 Metabolic scope, that is the difference between resting and maximal metabolic rates,
124 represents the energy (ATP) available for activity³¹. Resting metabolic rate represents the
125 energetic costs to maintain membrane potential, protein synthesis and other processes

126 occurring while the animal is at rest. Maximal metabolic rate reflects the maximal
127 mitochondrial and cardiovascular capacities ³¹. We measured (n = 12 lean fish, 14 obese, and
128 9 obese-lean fish for all measures of oxygen consumption) resting and maximal oxygen
129 consumption rates according to our previously published protocols ^{32,33} at 25°C.

130 Citrate synthase (CS) is a rate limiting enzyme in the TCA cycle, and its activity
131 reflects mitochondrial densities in tissue samples ³⁴. Fish (n = 8 fish per treatment group)
132 were anaesthetised in buffered ethyl 3-aminobenzoate methanesulfonate (MS222; 0.3 g l⁻¹;
133 Sigma-Aldrich, Castle Hill, Australia) and euthanized by decapitation. Dorsal (back) and
134 caudal (tail) skeletal muscle was extracted and immediately transferred to liquid nitrogen and
135 stored at -80°C. Muscle samples were homogenised (in a TissueLyser LT; Qiagen, Venlo,
136 Netherlands) in 9 volumes RIPA buffer (20mM TrisCl pH 7.5, 150 mM NaCl, 1 mM EDTA,
137 1 mM EGTA, 1% NP40, 1% sodium deoxycholate) and protease inhibitor cocktail
138 (cOmplete, EDTA-free; Roche Life Sciences, Germany) solution. Homogenate was further
139 diluted by a factor of 10 to a final 1:100 dilution. Following published protocols ³⁵, enzyme
140 activities were determined using a UV/visible spectrophotometer (Ultrospec 2100 Pro;
141 Biochrom, UK) with a temperature controlled cuvette holder. Assays were performed in
142 duplicate at 25°C.

143 Sustained swimming performance was measured (in n = 12 lean, 14 obese, and 9
144 obese-lean fish) as critical sustained swimming speed (U_{crit}) ³⁶ in a Blazka-type swimming
145 flume according to published protocols ³². The U_{crit} protocol uses an incremental increase in
146 speed (U_i) for predetermined time intervals (T_i) until fish are fatigued as a measure of
147 maximum locomotor capacity ³⁶.

148

149 *Muscle biomechanics*

150 Fish (n = 10 per treatment group) were euthanized via a blow to the head, and
151 transection of the spinal cord. The skin was removed and a section of rostral (anterior dorsal)
152 muscle fibres of 5 to 7 myotomes in length was dissected from one side of the fish in cooled
153 (<5°C) aerated fish Ringer's solution (composition in mmol l⁻¹: NaCl 115.7; sodium pyruvate
154 8.4; KCl 2.7; MgCl₂ 1.2; NaHCO₃ 5.6; NaH₂PO₄ 0.64; HEPES sodium salt 3.2; HEPES 0.97;
155 CaCl₂ 2.1; pH 7.4 at 20°C)³⁷. The spine was removed from most of the muscle preparation
156 leaving one myotome attached to the residual amount of spine at either end.

157 We conducted isometric studies to determine the twitch and tetanus kinetics of the
158 isolated muscle according to published protocols³⁸. We calculated rates of force production
159 as peak tetanic stress (force per cross-sectional area) divided by 2 x time to half peak tetanus,
160 and muscle relaxation as peak tetanic stress divided by 2 x time from last stimulus to half
161 relaxation as measures of the contractile performance of muscle.

162 We used the work loop technique to determine the power output (average of each
163 work loop cycle) of muscles during cyclical length changes¹⁵. Unlike fixed-length isometric
164 studies and fixed load isotonic studies of muscle performance, the work loop technique
165 allows measures of muscle power output under length and activation changes that are
166 generally more indicative of *in vivo* contractile performance³⁹. In the absence of *in vivo*
167 strain (length change) data for rostral muscle in zebrafish, each muscle preparation was
168 subjected to a set of four sinusoidal length changes symmetrical around the length found to
169 generate maximal twitch force. *In vivo* rostral muscle length changes have been found to
170 approximate a sinusoidal length change waveform in fish undergoing steady swimming, with
171 the primary function of such muscle to produce power⁴⁰. The muscle was stimulated using
172 the stimulation amplitude and stimulation frequency found to yield maximal isometric force.
173 Electrical stimulation and length changes were controlled via a data acquisition board
174 (KUSB3116, Keithley Instruments, Ohio, USA) and a custom-designed program developed

175 via TestPoint software (CEC Testpoint version 7, Measurement Computing, Norton,
176 Massachusetts, USA). Muscle force was plotted against muscle length for each cycle to
177 generate a work loop, the area of which equated to the net work produced by the muscle
178 during the cycle of length change ⁴¹. Instantaneous power output was calculated for every
179 data point in each work loop (2,000 data points per work loop) by multiplying instantaneous
180 velocity by instantaneous force. These instantaneous power output values were then averaged
181 to generate an average net power output for each work loop cycle. Every 5 minutes, the
182 muscle was subjected to a further set of four work loop cycles with length change cycle
183 frequency (between 3 and 22 Hz), strain, stimulation duration and stimulation phase
184 parameters being altered in between each set until maximum net work was achieved at each
185 cycle frequency and maximal power output had been determined.

186 Every fourth or fifth set of work loop cycles was used as a control run whereby a
187 fixed set of strain and stimulation parameters were repeated regularly throughout the
188 experiment to monitor underlying changes in the performance of the muscle over time. On
189 average the net mean muscle power output per cycle, produced in control runs, decreased by
190 8.7% over the time course of each experiment. Therefore, the power produced by each
191 preparation was corrected to the control run that yielded the highest power output (average
192 power per cycle), assuming that alterations in power generating ability were linear over time
193 between control runs.

194 After a further 5 minute rest, fatigue resistance was determined by subjecting the
195 muscle preparation to a series of tetani, each of 150 ms stimulation duration, at a rate of one
196 tetanus per second for 25 s. For each muscle, fatigue resistance was calculated as the maximal
197 force produced in the 25th tetanus as a percentage of the maximal force produced in the 1st
198 tetanus for the same muscle. Ten minutes after the fatigue run each preparation was
199 stimulated to produce a further tetanus to determine recovery from the fatigue run. The mean

200 recovery of all 30 muscle preparations was 81.1% indicating that reversible fatigue had been
201 induced.

202 At the end of the muscle mechanics experiments, bone and connective tissue were
203 removed and each muscle preparation was blotted on absorbent paper to remove excess
204 Ringer's solution. Wet muscle mass was determined to the nearest 0.1 mg using an electronic
205 balance (Sartorius, Australia). Mean muscle cross-sectional area was calculated from muscle
206 length and mass assuming a density of 1060 kg m^{-3} ⁴². The overall mean cross-sectional area
207 \pm s.e. of all 30 muscle preparations was $2.65 \pm 0.17 \text{ mm}^2$. Maximum isometric muscle stress
208 (kN m^{-2}) was then calculated for each tetanic response as the maximum tetanic force within
209 that response divided by mean cross-sectional area. Normalised muscle power output (W kg^{-1})
210 was calculated as average power output per length change cycle divided by wet muscle
211 mass.

212

213 *Myosin heavy chain concentrations*

214 We prepared tissue homogenates as described above for measures of citrate synthase
215 activity. The identification and quantification of slow and fast myosin heavy chain (MHC)
216 isoforms was performed by capillary electrophoresis in a "Wes" Simple Western System
217 (ProteinSimple, CA, USA) following the manufacturer's instructions. The antibodies (all
218 from Developmental Studies Hybridoma Bank, University of Iowa, USA) we used were:
219 EB165 to determine fast MHC concentrations; BA-F8 to determine slow MHC
220 concentrations; 12G10 (α -tubulin) as internal control. We expressed normalised MHC
221 concentrations by dividing MHC peaks by α -tubulin peaks measured for the same sample on
222 the same plate. The concentrations of protein extracts was determined using a bicinchoninic
223 acid assay kit (Sigma-Aldrich, Castle Hill, Australia) following the manufacturer's
224 instructions.

225

226 *Statistical analyses*

227 We analysed data with permutational tests for linear models in the package *lmPerm*⁴³
228 in R⁴⁴. Permutational analyses do not make assumptions about underlying data distributions,
229 but use the data per se to infer significant differences. This approach is preferable to
230 parametric tests, especially for sample sizes that are small relative to the total population of
231 all possible samples⁴⁵. We analysed all dependent variables (BMI, U_{crit} , metabolic rates,
232 muscle mechanics, MHC concentrations and enzyme activities) with treatment (levels: lean,
233 obese, obese-lean) as factor. In the analysis comparing power output at different cycle
234 frequencies, we use treatment as fixed factor, and we used fish id as a random factor within
235 which we nested the different cycle frequencies to account for repeated measures of the same
236 muscle preparation at different cycle frequencies. In analyses of U_{crit} (in $m\ s^{-1}$) we used body
237 length as covariate, but we show data in units of body lengths s^{-1} . In case of significant
238 results, we used pair-wise permutational tests for post hoc comparisons, and we used $p < 0.05$
239 to indicate significant differences between treatment groups. Sample sizes were based on the
240 power we achieved using similar techniques on zebrafish in past experiments^{38,46}.

241

242 **Results**

243 *Obese fish had greater body mass index*

244 Body mass indices differed significantly between treatments ($p < 0.001$; Fig. 1), and
245 lean and obese-lean fish had significantly lower body mass indices than obese fish (both $p <$
246 0.001), but there was no difference in body mass index between lean and obese-lean fish ($p =$
247 0.63). There was no difference in body mass index between the obese and the obese-lean
248 groups ($p = 0.82$) just before the start of diet restriction of the obese-lean group when the
249 feeding regime was switched from feeding three time per day to once per day.

250

251 *Obesity caused reversible decreases in metabolic scope independently from citrate synthase*
252 *activity*

253 Resting metabolic rates differed significantly between treatments ($p = 0.0068$; Fig.
254 2A). Lean and obese-lean fish had similar resting metabolic rates ($p = 0.98$), and the rates of
255 both groups were lower than that of the obese fish ($p < 0.001$ and $p = 0.040$, respectively for
256 lean and obese-lean). Maximal metabolic rates also differed between treatments ($p = 0.035$;
257 Fig. 1B), and obese fish had significantly lower maximal metabolic rates than lean fish ($p =$
258 0.030), but there were no differences between obese-lean and obese ($p = 0.72$) or lean ($p =$
259 0.13) fish. These responses of resting and maximal metabolic rates led to differences in
260 metabolic scope between treatments ($p = 0.0026$; Fig. 2C), and lean fish had higher metabolic
261 scope than obese fish ($p = 0.0060$), but obese-lean fish did not differ from either of the other
262 groups ($p = 0.13$ and $p = 0.75$, respectively).

263 Citrate synthase activity, an indicator of mitochondrial density and metabolic
264 capacity, did not differ between the treatment groups ($p = 0.43$; Fig. 2D).

265

266 *Obesity caused irreversible changes in locomotor performance and muscle contractile*
267 *properties*

268 There were significant differences in U_{crit} between treatments ($p = 0.0034$; Fig. 3A).
269 Compared to lean fish, swimming performance was significantly lower in obese ($p = 0.044$)
270 and in obese-lean ($p = 0.0066$) fish, but there was no difference between the latter two groups
271 ($p = 0.27$).

272 There were significant effects of treatment on muscle isometric stress (force per unit
273 area; $p = 0.023$; Fig. 3F) and muscle work-loop power output (power produced per muscle
274 mass; $p = 0.016$; Fig. 3C), and both were lowest in obese-lean fish (lean vs obese-lean: stress

275 $p = 0.0098$, power $p < 0.001$; obese vs obese-lean: stress $p = 0.029$, power $p = 0.011$). There
276 was a reduction in power and stress in obese fish compared to lean fish, but this was
277 significant at a one-tailed probability only (stress $p = 0.092$, power $p = 0.086$; Fig. 3C and D).
278 Work loop shapes indicated that most of the difference in normalized power output between
279 lean and obese-lean fish was due to lean fish generating a higher peak stress in the work loop
280 and maintaining higher stress during shortening (Fig 3D). Differences in power output
281 between treatments were apparent only at higher cycle frequencies (interaction between
282 treatment and cycle frequency $p < 0.0001$; Fig. 3E). At cycle frequencies of 12 Hz and above,
283 power output was significantly lower in obese-lean fish compared to lean fish (all $p < 0.05$),
284 and obese-lean fish produced less power than obese fish at cycle frequencies of 14 Hz and
285 above (all $p < 0.05$; Fig. 3E). Power output of obese fish was variable and we detected no
286 differences between lean and obese fish (all $p > 0.2$; Fig. 3E).

287 Muscle activation rates were significantly different at a one-tailed probability only (p
288 $= 0.074$; Fig. 3G). Muscle relaxation rates differed significantly between treatments ($p =$
289 0.02 ; Fig. 3H), and relaxation rate was significantly faster in muscle of lean fish compared to
290 obese-lean fish ($p = 0.012$); obese fish differed from lean fish with a one-tailed probability (p
291 $= 0.067$), but there was no difference in relaxation rate between obese and obese-lean fish.
292 There were no differences between treatments in muscle fatigue ($p = 0.98$; Fig. 3B).

293

294 *Myosin heavy chains changed irreversibly with obesity*

295 Obesity treatment had a significant effect on (normalised) slow myosin heavy chain
296 concentrations ($p < 0.001$; Fig. 4A). Lean fish had significantly greater concentrations than
297 obese ($p = 0.012$), and obese-lean ($p = 0.0062$) fish, but there was no difference between the
298 latter two groups ($p = 0.75$). Similarly, concentrations of fast myosin heavy chains changed
299 with treatment ($p = 0.044$; Fig. 4B), and compared to lean fish obese-lean fish had

300 significantly lower concentrations of fast myosin heavy chains ($p = 0.025$), but the decrease
301 in obese fish was significant at a 1-tailed probability only ($p = 0.078$). Muscle composition
302 changed with treatment (treatment effect $p = 0.0096$, Fig. 4C), and the ratio between
303 slow:fast myosin heavy chains was significantly lower in obese ($p = 0.029$) and obese-lean
304 fish ($p = 0.010$) than in lean fish, but obese fish were not different from obese-lean fish ($p =$
305 0.96).

306

307 **Discussion**

308 We have shown that declines in metabolic scope in obese individuals are reversed by
309 weight loss, but declines in muscle contractile function and locomotion are not. These results
310 indicate that weight gain and loss influence metabolic responses directly, but that the effect
311 of obesity on muscle phenotypes is not mediated directly by changes in body mass (BMI).
312 The implication of our finding is that weight loss alone may be an insufficient treatment for
313 obese pathologies.

314 Zebrafish are well established now in the literature as a model for obesity^{6,7,47},
315 metabolic disease⁴⁸ and exercise⁴⁹⁻⁵¹. Overfeeding in zebrafish led to rapid weight gain and
316 a significant increase in body mass index (1.1-1.3 fold) compared to control fish after 1-2
317 weeks⁷. Similar to the effects of weight gain and obesity in humans, the increase in body
318 mass resulted in pathophysiological conditions such as hypertriglyceridemia and
319 hepatosteatosis^{7,52}. Hence, that level of weight gain (>1.1-1.3 increase from lean body mass
320 index) may be defined functionally as obese^{53,54}. As in mice and humans, disruption of the
321 adipostat system caused obesity in zebrafish⁶, and leptin receptor deficiency in zebrafish
322 disrupted glucose homeostasis, but it did not cause hyperphagia⁵⁵. Exercise training in
323 zebrafish increased muscle mass⁵¹, myogenin levels, and shifted skeletal muscle to a slower

324 and more aerobic fibre type ⁵⁶. These responses are broadly similar to those of other
325 vertebrates ^{2,57}, which makes zebrafish a good exercise model for biomedical research ⁵⁰.

326 The (patho)physiological similarities between zebrafish and humans, combined with
327 the lower cost and increased tractability of conducting experimental and screening studies on
328 zebrafish compared to rodents or humans^{47,48,58,59} mean that zebrafish have increasing
329 translational impact ^{59,60}. Zebrafish are particularly suitable for studies on muscle function and
330 exercise because the methodologies to determine muscle and locomotor performance are well
331 established in fish ⁶¹⁻⁶³. We recently optimised isometric techniques to measure muscle
332 performance in zebrafish ³⁸, which we extended here to include the work-loo technique.
333 These techniques are particularly powerful in a zebrafish model, because here it utilises most
334 of the locomotory muscle assembly to provide a functional measure of muscle performance,
335 which is more realistic than approaches that use only single fibres from biopsies as is the case
336 for human studies. Hence, for our study, as well as for many others⁴⁷, zebrafish were a
337 superior model than humans, in terms of quality of data, sample sizes, and practicality in
338 terms of manipulating weight gain and weight loss under controlled experimental conditions.

339 Chronic feeding on high-energy diets and a sedentary lifestyle lead to an imbalance in
340 glucose metabolism and insulin signaling, which can lead to obesity and metabolic diseases⁴.
341 A mechanism by which these effects can be mediated is the action of the SIRT1, which in
342 association with AMPK activity regulates fatty acid oxidation and energy homeostasis ¹⁰.
343 SIRT1 levels are increased by caloric restriction and are decreased by overfeeding ⁴.
344 Adiponectin, the levels of which decrease with obesity but are restored by weight loss ²⁶,
345 stimulates the SIRT1/AMKP axis ⁶⁴. Together, the actions of these molecules provide a
346 mechanistic link between excessive feeding and obesity on the one hand, and metabolic
347 dysfunction on the other ⁶⁵, and may explain why metabolic dysfunction is reversible by
348 decreased feeding and weight loss. Our finding that obesity-induced decreases in metabolic

349 scope are reversible by reduced feeding and weight loss are similar to responses from
350 mammals. The decrease in (mass specific) maximal metabolic rates was expected from
351 obesity-induced metabolic dysfunction, and from the increase in adiposity in obese
352 individuals. However, the decreases in maximal metabolic rates were not associated with
353 decreased citrate synthase activities, which indicates that mitochondrial densities in muscle
354 were not altered by obesity ³⁴. The observed increases in resting metabolic rate may be due to
355 increased inflammation and its attendant increase in resting metabolic demand ⁶⁶, but this
356 suggestion should be verified in a zebrafish model.

357 Surprisingly, the obesity-induced decreases in muscle contractile function and
358 locomotor capacity were not reversible by weight loss in our zebrafish. High fat diet caused a
359 shift in myosin heavy chains towards faster isoforms in rhesus monkeys, and that shift was
360 partly reversed with resveratrol, a drug that stimulates the SIRT1 pathway and promotes
361 mitochondrial proliferation ⁶⁷. Decreases in adiponectin and its receptor AdipoR1 can also
362 decrease oxidative type I myofibres ⁶⁴. However, if adiponectin and SIRT1 levels are restored
363 by weight loss, as suggested in the literature, some other mechanisms must regulate muscle
364 function and myosin heavy chain expression in our zebrafish. Our data indicate that muscle
365 of obese and obese-lean fish had low myofibrillar density (low MHC concentrations), and
366 this decrease can explain the decreases in muscle stress and power output. The increasing
367 difference in power output between treatments with increasing cycle frequency confirm this
368 suggestion, because the effect of low myofibrillar density would be particularly pronounced
369 as muscle works harder and at higher cycle frequencies. The cycle frequencies of the work
370 loop assays are proportional to tail beat frequencies in swimming fish ¹⁵, and tail beat
371 frequencies are proportional to swimming speed ⁶⁸. Hence, the reduction in myosin heavy
372 chain concentrations and power output at high cycle frequencies can explain the decreases in
373 U_{crit} we observed in obese and obese-lean individuals. The reduction in MHC concentration,

374 and the shift from slow to fast MHC could be due to impaired signalling pathways that
375 mediate expression of muscle proteins. For example, expression of calcium handling and
376 contractile proteins in skeletal muscle is regulated by the interaction between myocyte
377 enhancer factor 2 (MEF2) and histone deacetylases (HDAC) ². Obesity can lead to a
378 disruption of the transcriptional regulation of muscle phenotypes, thereby leading to
379 decreased muscle mass and strength ⁶⁹, which provides a explanatory model for the changes
380 in MHC levels we observed that can be tested in zebrafish and other obesity models.

381 Tail beat frequency, and hence swimming performance, is sensitive to calcium release
382 and resequestration into the sarcoplasmic reticulum ⁶⁸. Obesity can alter calcium (Ca²⁺)
383 concentrations ⁷⁰ and reduce sarco-endoplasmic reticulum Ca²⁺-ATPase (SERCA) activity ⁷¹,
384 which provides a second avenue by which obesity can constrain locomotion at higher speeds
385 (i.e. cycle frequencies - tail beat frequencies). Muscle contraction is mediated by the release
386 of Ca²⁺ from the sarcoplasmic reticulum following neural stimulation of dihydropyridine
387 receptors and their interaction with ryanodine receptors ⁷². Free Ca²⁺ mediates muscle
388 contraction by binding to troponin in a concentration-dependent manner. Muscle relaxation is
389 mediated by re-sequestration of Ca²⁺ into the sarcoplasmic reticulum via SERCA ⁵⁷.
390 Disruption of Ca²⁺ dynamics will attenuate muscle contractile properties ⁵⁷ and decrease
391 locomotor performance ⁶⁸. The reduction in relaxation rate indicates that obesity reduced
392 SERCA activity and thereby slowed re-sequestration of Ca²⁺ into the sarcoplasmic reticulum
393 and muscle relaxation. Similarly, the (one-tailed) decrease in activation rate suggests that the
394 rate of Ca²⁺ release from the sarcoplasmic reticulum is reduced following stimulation.
395 Depletion of Ca²⁺ stores in the sarcoplasmic reticulum can also reduce stress and power
396 output ⁵⁷. However, store depletion is unlikely, because fatigue resistance, which is at least
397 partly determined by store depletion ⁷³, was not affected by obesity.

398 Zebrafish are an excellent model to test obesity-induced changes on skeletal muscle,

399 because it is easier to isolate the effects of obesity on muscle per se. In terrestrial animals,
400 including humans, obesity increases the in vivo strength of postural ‘antigravity’ muscles as a
401 result of a training effect from the increased load during standing and locomotion ⁷⁴, which
402 can obscure the effects of obesity on locomotor muscle. Our data are important because we
403 show that the effects of obesity persist beyond weight loss. Weight loss is an essential
404 intervention for obesity, but our data indicate that it is not sufficient to restore healthy, pre-
405 obese phenotypes. The average lifespan of zebrafish is around 5% that of humans ⁷⁵. Hence,
406 the period of diet restriction (4-5 weeks) in our experiments represents a reasonably long
407 time in human terms. An important outstanding question now is whether the observed
408 changes, such as myosin heavy chain concentrations and composition, can revert back to pre-
409 obesity levels. Even though there can be a training effect of postural muscle as a result of
410 supporting larger mass ⁷⁴, obesity leads to reductions in motor control ⁷⁶ and it is often
411 associated with sedentary lifestyles ⁷⁷ so that the mass-induced training effect would be
412 minimised ⁷⁸. Exercise intervention could be effective in restoring muscle function as well as
413 weight loss ⁷⁹. An interesting future direction will be to determine the link between
414 transcriptional regulation of muscle phenotypes and changes in the contractile apparatus of
415 skeletal muscle during obesity and following weight loss in both the zebrafish model and in
416 humans directly. Understanding the role of exercise in influencing these pathways during or
417 following weight loss could lead to developing effective programs to reverse the negative
418 effects of obesity on muscle function and locomotor capacity.

419

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423

424 **Conflict of interest**

425 The authors declare no conflict of interest.

426

427 **Data accessibility**

428 Data will be deposited in Dryad upon acceptance.

429

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652 **Figure captions**

653 **Figure 1** Body mass indices of the treatment groups. Lean (L) and obese-lean (OL; obese
654 fish that underwent weight loss) fish had similar body mass indices (BMI), which were
655 significantly lower than those of obese (O) fish. The BMI of the obese group was not different
656 from that of the obese-lean group just before diet restriction when the feeding regime was
657 switched from three times to once per day (OL/O). Means \pm s.e. are shown, and letters above
658 bars indicate significant differences.

659

660 **Figure 2** Metabolic responses of zebrafish to obesity and weight loss. Obese (O) fish had
661 significantly greater resting metabolic rates than lean (L) or obese-lean (OL) fish (A), but
662 maximal metabolic rates of obese fish were lower than in lean fish (B). Maximal metabolic
663 rates of obese-lean fish were not different from lean or obese fish. Metabolic scope was
664 reduced in obese fish (C), but at least partly restored after weight loss in obese-lean fish.
665 Citrate synthase activity, an indicator of mitochondrial density, did not differ significantly
666 between treatment groups (D). Means \pm s.e. are shown, and letters above bars indicate
667 significant differences. $n = 12$ lean, 14 obese, and 9 lean-obese fish for all metabolic rate
668 measures, and $n = 8$ fish per treatment group for citrate synthase activity.

669

670 **Figure 3** Muscle mechanics and locomotor performance in response to obesity and weight
671 loss. Sustained swimming performance (U_{crit} ; A), isometric stress (force per unit area, F),
672 dynamic muscle power output determined by the work-loop technique (C), and muscle
673 relaxation rates (H) were lower in obese (O) individuals compared to lean (L) fish, and

674 stayed at a reduced level even after weight loss (obese-lean, OL). An example of a typical
675 work loop shape (D) demonstrates that muscle of lean fish (broken line) produced greater
676 stress and maintained stress to a greater extent during shortening (decreasing strain)
677 compared to obese-lean fish (solid line). Differences in muscle power output between
678 treatments were apparent at high cycle frequencies (E; significant differences indicated by an
679 asterisk). Activation rate (G) showed similar reductions in obese and obese-lean individuals,
680 but the differences were significant at a one-tailed probability only. There was no effect of
681 treatment on muscle fatigue (B). Means \pm s.e. are shown, and letters above bars indicate
682 significant differences. An hash next to a letter (e.g. a[#]) indicates differences with a one-
683 tailed probability. For U_{crit} , n = 12 lean, 14 obese, and 9 obese-lean fish, and n = 10 fish per
684 treatment group for measures of muscle mechanics.

685

686 **Figure 4** Myosin heavy chain concentrations in response to obesity and weight loss. Slow
687 (A) and fast (B) myosin heavy chain concentrations (MHC; normalised to α -tubulin) were
688 significantly lower in obese (O) and obese-lean (OL) individuals compared to lean controls
689 (L). The slow:fast MHC ratio was lower in obese and obese-lean individuals, indicating a
690 shift in muscle composition (C). Means \pm s.e. are shown, and letters above bars indicate
691 significant differences. An hash next to a letter (e.g. a[#]) indicates differences with a one-
692 tailed probability. N = 6 individuals for each treatment group.

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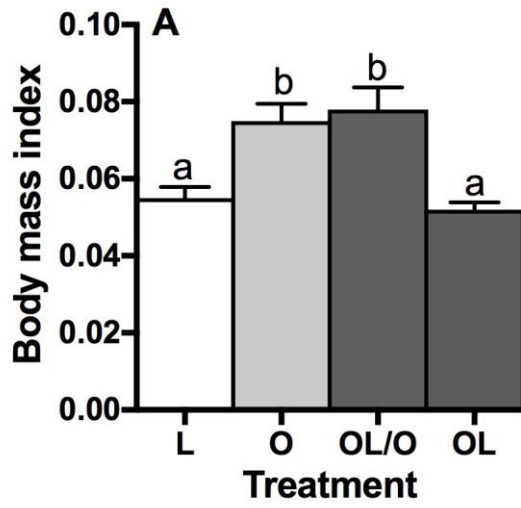
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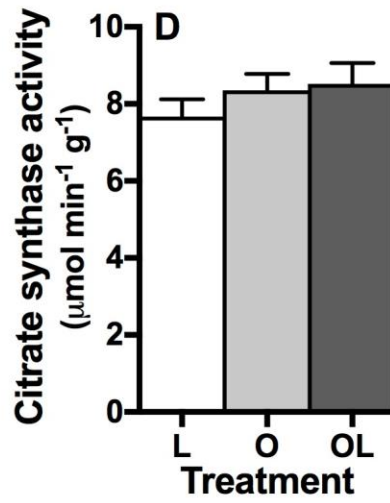
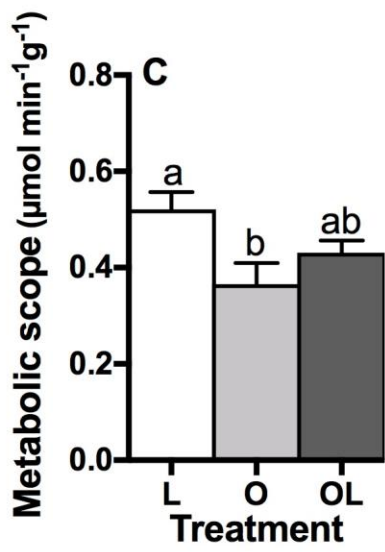
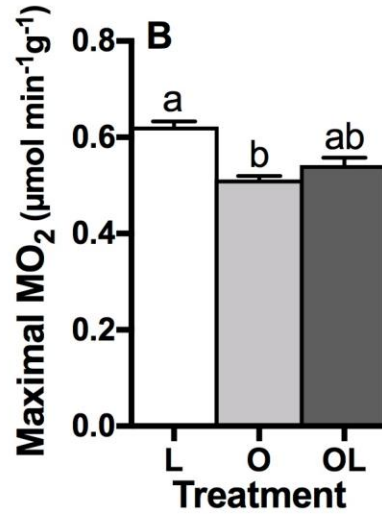
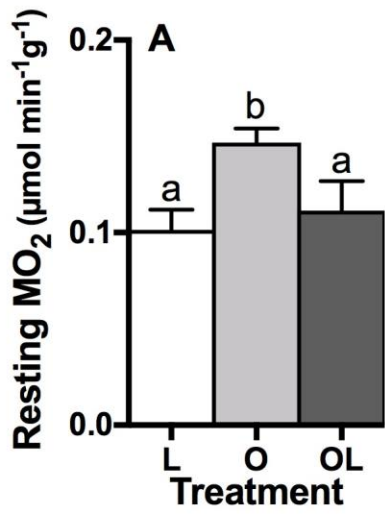
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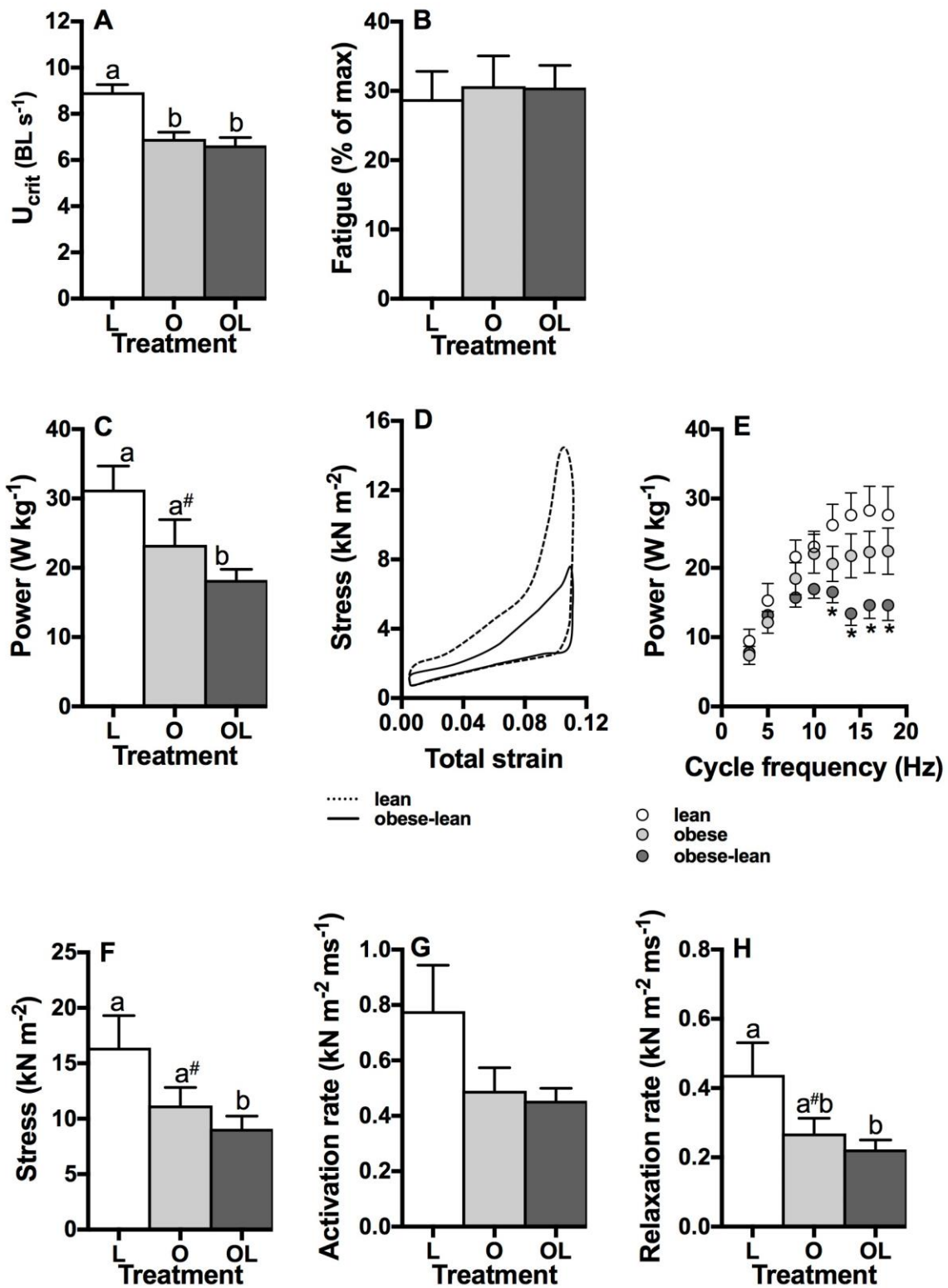
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Fig 3

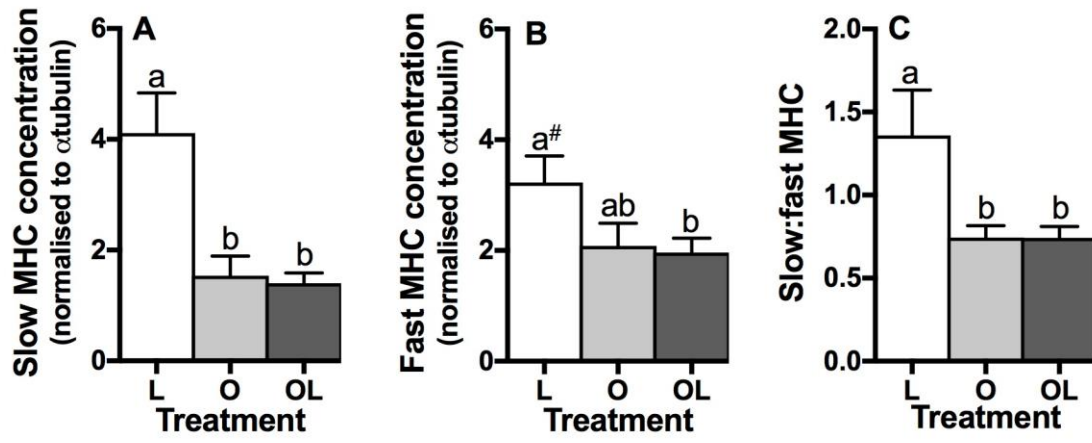


Fig 4