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

Frank Seebacher[1*], Jason Tallis[2], Kate McShea[1], Rob S. James[2]

[1]*School of Life and Environmental Sciences A08 University of Sydney, Science Road, Sydney, NSW, 2006, Australia*

[2]*Centre for Applied Biological and Exercise Sciences, Faculty of Health and Life Sciences, Coventry University, Coventry, CV1 5FB, U.K*

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*author for correspondence: frank.seebacher@sydney.edu.au

Conflict of Interest

The authors declare no conflict of interest.

Abstract

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

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

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

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

Introduction

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

Many signalling pathways associated with energy homeostasis are conserved among vertebrates and are similar in a number of model species, including humans, rodents, and zebrafish [6,7]. One of the principal mediators of obesity-induced metabolic dysfunction is a reduction in concentration and activity of the sirtuin SIRT1[4,8]. SIRT1 is a NAD^+-dependent histone deacetylase that promotes expression of a range of metabolic regulators such as PGC-1alpha [9], and thereby regulates mitochondrial function [10]. Mice lacking SIRT1 had reduced activity and reduced rates of oxygen consumption, leading to an overall decrease in energy expenditure [11]. Conversely, increased expression of SIRT1 led to increased energy expenditure[12]. Obesity-induced mitochondrial dysfunction in skeletal muscle manifests as decreased TCA cycle (citrate synthase) activity and electron transport chain flux [13]. It would be expected therefore that this reduction in maximal metabolic capacities would lead to a

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

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

Weight loss reversed obesity-induced increases in pro-inflammatory proteins [25], reductions in adiponectin levels[26], impaired lymphatic function [27], metabolic dysfunction [28,29], and reduction in slow type I myosin heavy chains [17]. However, at least with respect to kidney function it is not clear whether the effects of obesity are reversible by weight loss [30]. Overall, it may be expected that obesity-induced declines in physiological function are reversed by weight loss. However, the physiological effects of obesity are so complex that it is difficult to extrapolate between physiological systems. Hence, we tested whether obesity-induced impacts on muscle and locomotor function are reversible by weight loss.

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

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

Materials and Methods

Study animals and treatments

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

Metabolism and swimming performance

Metabolic scope, that is the difference between resting and maximal metabolic rates, represents the energy (ATP) available for activity [31]. Resting metabolic rate represents the energetic costs to maintain membrane potential, protein synthesis and other processes

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

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

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

Muscle biomechanics

Fish (n = 10 per treatment group) were euthanized via a blow to the head, and transection of the spinal cord. The skin was removed and a section of rostral (anterior dorsal) muscle fibres of 5 to 7 myotomes in length was dissected from one side of the fish in cooled (<5°C) aerated fish Ringer's solution (composition in mmol l^{-1}: NaCl 115.7; sodium pyruvate 8.4; KCl 2.7; $MgCl_2$ 1.2; $NaHCO_3$ 5.6; NaH_2PO_4 0.64; HEPES sodium salt 3.2; HEPES 0.97; $CaCl_2$ 2.1; pH 7.4 at 20°C) [37]. The spine was removed from most of the muscle preparation leaving one myotome attached to the residual amount of spine at either end.

We conducted isometric studies to determine the twitch and tetanus kinetics of the isolated muscle according to published protocols [38]. We calculated rates of force production as peak tetanic stress (force per cross-sectional area) divided by 2 x time to half peak tetanus, and muscle relaxation as peak tetanic stress divided by 2 x time from last stimulus to half relaxation as measures of the contractile performance of muscle.

We used the work loop technique to determine the power output (average of each work loop cycle) of muscles during cyclical length changes [15]. Unlike fixed-length isometric studies and fixed load isotonic studies of muscle performance, the work loop technique allows measures of muscle power output under length and activation changes that are generally more indicative of *in vivo* contractile performance [39]. In the absence of *in vivo* strain (length change) data for rostral muscle in zebrafish, each muscle preparation was subjected to a set of four sinusoidal length changes symmetrical around the length found to generate maximal twitch force. *In vivo* rostral muscle length changes have been found to approximate a sinusoidal length change waveform in fish undergoing steady swimming, with the primary function of such muscle to produce power [40]. The muscle was stimulated using the stimulation amplitude and stimulation frequency found to yield maximal isometric force. Electrical stimulation and length changes were controlled via a data acquisition board (KUSB3116, Keithley Instruments, Ohio, USA) and a custom-designed program developed

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

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

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

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

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

Myosin heavy chain concentrations

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

Statistical analyses

We analysed data with permutational tests for linear models in the package lmPerm [43] in R [44]. Permutational analyses do not make assumptions about underlying data distributions, but use the data per se to infer significant differences. This approach is preferable to parametric tests, especially for sample sizes that are small relative to the total population of all possible samples [45]. We analysed all dependent variables (BMI, U_{crit}, metabolic rates, muscle mechanics, MHC concentrations and enzyme activities) with treatment (levels: lean, obese, obese-lean) as factor. In the analysis comparing power output at different cycle frequencies, we use treatment as fixed factor, and we used fish id as a random factor within which we nested the different cycle frequencies to account for repeated measures of the same muscle preparation at different cycle frequencies. In analyses of U_{crit} (in m s^{-1}) we used body length as covariate, but we show data in units of body lengths s^{-1}. In case of significant results, we used pair-wise permutational tests for post hoc comparisons, and we used $p < 0.05$ to indicate significant differences between treatment groups. Sample sizes were based on the power we achieved using similar techniques on zebrafish in past experiments[38,46].

Results

Obese fish had greater body mass index

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

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

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

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

Obesity caused irreversible changes in locomotor performance and muscle contractile properties

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

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

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

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

Myosin heavy chains changed irreversibly with obesity

Obesity treatment had a significant effect on (normalised) slow myosin heavy chain concentrations ($p < 0.001$; Fig. 4A). Lean fish had significantly greater concentrations than obese ($p = 0.012$), and obese-lean ($p = 0.0062$) fish, but there was no difference between the latter two groups ($p = 0.75$). Similarly, concentrations of fast myosin heavy chains changed 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 disease [48] and exercise [49-51]. 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 weeks [7]. 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 zebrafish[6], and leptin receptor deficiency in zebrafish
322 disrupted glucose homeostasis, but it did not cause hyperphagia [55]. Exercise training in
323 zebrafish increased muscle mass[51], myogenin levels, and shifted skeletal muscle to a slower

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

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

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

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

Surprisingly, the obesity-induced decreases in muscle contractile function and locomotor capacity were not reversible by weight loss in our zebrafish. High fat diet caused a shift in myosin heavy chains towards faster isoforms in rhesus monkeys, and that shift was partly reversed with resveratrol, a drug that stimulates the SIRT1 pathway and promotes mitochondrial proliferation [67]. Decreases in adiponectin and its receptor AdipoR1 can also decrease oxidative type I myofibres [64]. However, if adiponectin and SIRT1 levels are restored by weight loss, as suggested in the literature, some other mechanisms must regulate muscle function and myosin heavy chain expression in our zebrafish. Our data indicate that muscle of obese and obese-lean fish had low myofibrillar density (low MHC concentrations), and this decrease can explain the decreases in muscle stress and power output. The increasing difference in power output between treatments with increasing cycle frequency confirm this suggestion, because the effect of low myofibrillar density would be particularly pronounced as muscle works harder and at higher cycle frequencies. The cycle frequencies of the work loop assays are proportional to tail beat frequencies in swimming fish [15], and tail beat frequencies are proportional to swimming speed [68]. Hence, the reduction in myosin heavy chain concentrations and power output at high cycle frequencies can explain the decreases in 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 (Ca^{2+})
383 concentrations [70] and reduce sarco-endoplasmic reticulum Ca^{2+}-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 Ca^{2+} from the sarcoplasmic reticulum following neural stimulation of dihydropyridine
387 receptors and their interaction with ryanodine receptors [72]. Free Ca^{2+} mediates muscle
388 contraction by binding to troponin in a concentration-dependent manner. Muscle relaxation is
389 mediated by re-sequestration of Ca^{2+} into the sarcoplasmic reticulum via SERCA [57].
390 Disruption of Ca^{2+} 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 Ca^{2+} into the sarcoplasmic reticulum
393 and muscle relaxation. Similarly, the (one-tailed) decrease in activation rate suggests that the
394 rate of Ca^{2+} release from the sarcoplasmic reticulum is reduced following stimulation.
395 Depletion of Ca^{2+} 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

Conflict of interest

The authors declare no conflict of interest.

Data accessibility

Data will be deposited in Dryad upon acceptance.

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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 that 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 time to once per day (OL/O). Means ± s.e. are shown, and letters above
658 bars indicate significant differences.

659

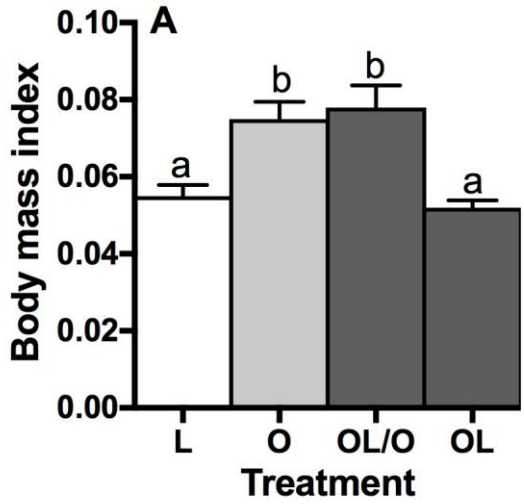
660 **Figure 2** Metabolic responses of zebrafish to obesity and weight loss. Obese (O) fish had
661 significantly greater resting metabolic rates than lean (L) or obese-lean (OL) fish (A), but
662 maximal metabolic rates of obese fish were lower than in lean fish (B). Maximal metabolic
663 rates of 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 ± 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

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

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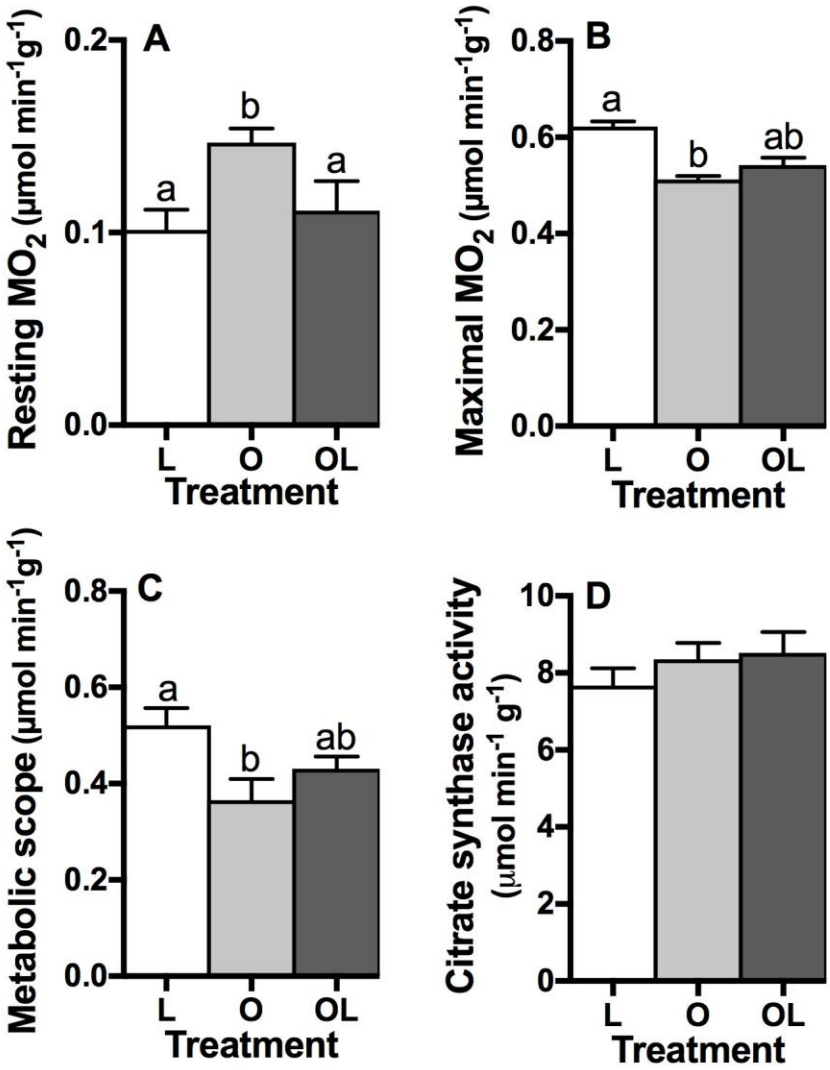
Treatment

Fig 1



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704 Fig 2

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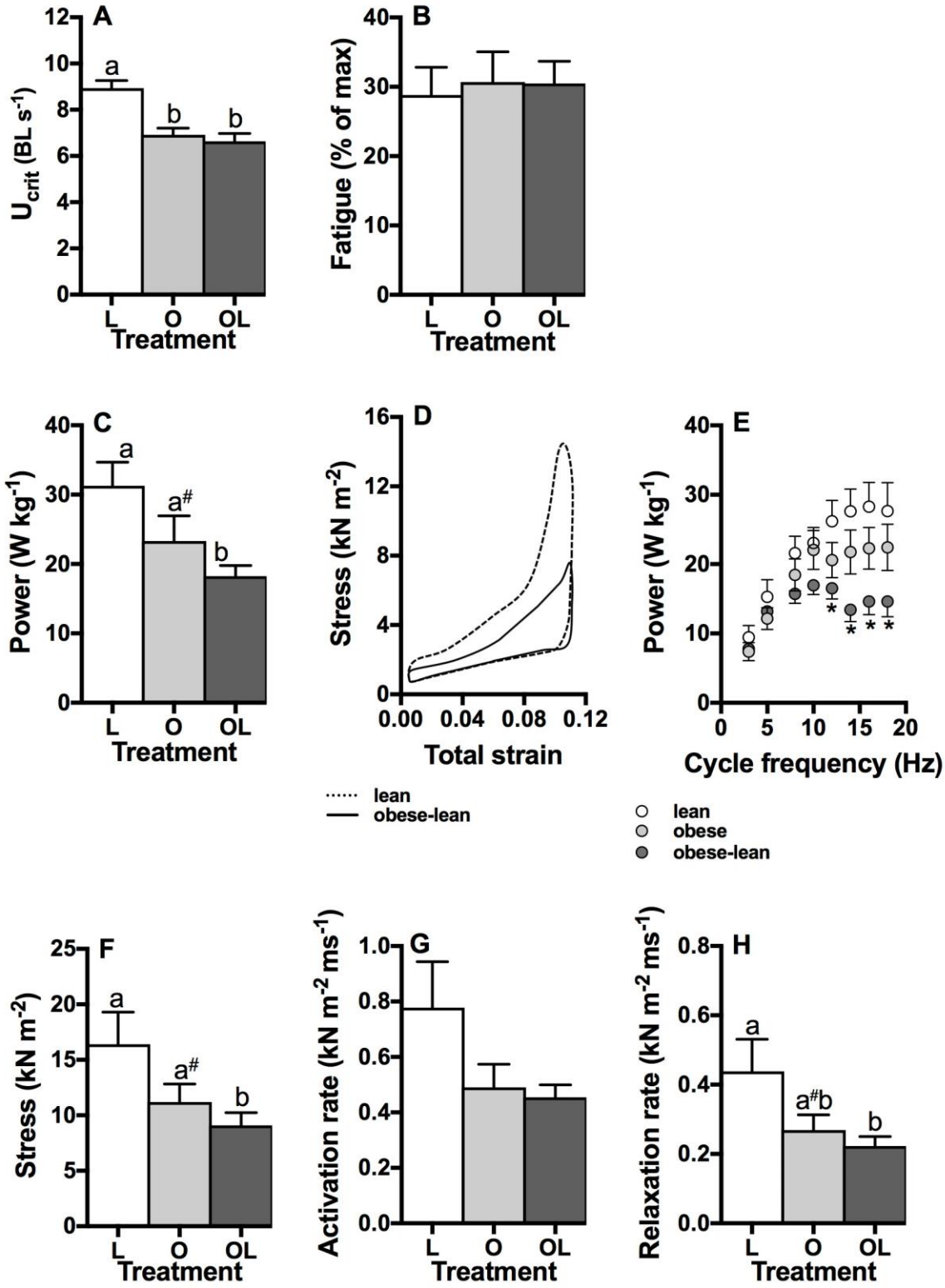
A
U_{crit} (BL s^{-1})
a
b
b
L
O
OL
Treatment
B
Fatigue (% of max)
L
O
OL
Treatment
C
Power (W kg^{-1})
a
a#
b
L
O
OL
Treatment
D
Stress (kN m^{-2})
0.00
0.04
0.08
0.12
Total strain
lean
obese-lean
E
Power (W kg^{-1})
0
5
10
15
20
Cycle frequency (Hz)
lean
obese
obese-lean
F
Stress (kN m^{-2})
a
a#
b
L
O
OL
Treatment
G
Activation rate (kN m^{-2} ms^{-1})
L
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Treatment
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Relaxation rate (kN m^{-2} ms^{-1})
a
a#b
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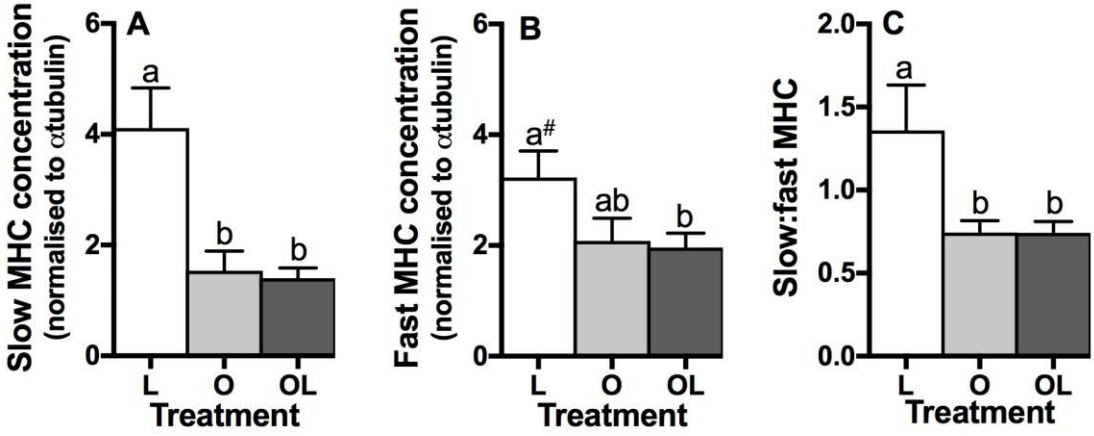
O
OL
Treatment

706

Fig 3


A
Slow MHC concentration
(normalised to αtubulin)
6
4
2
0
a
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b
L
O
OL
Treatment
B
Fast MHC concentration
(normalised to αtubulin)
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a#
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b
L
O
OL
Treatment
C
Slow:fast MHC
2.0
1.5
1.0
0.5
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a
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L
O
OL
Treatment

Fig 4