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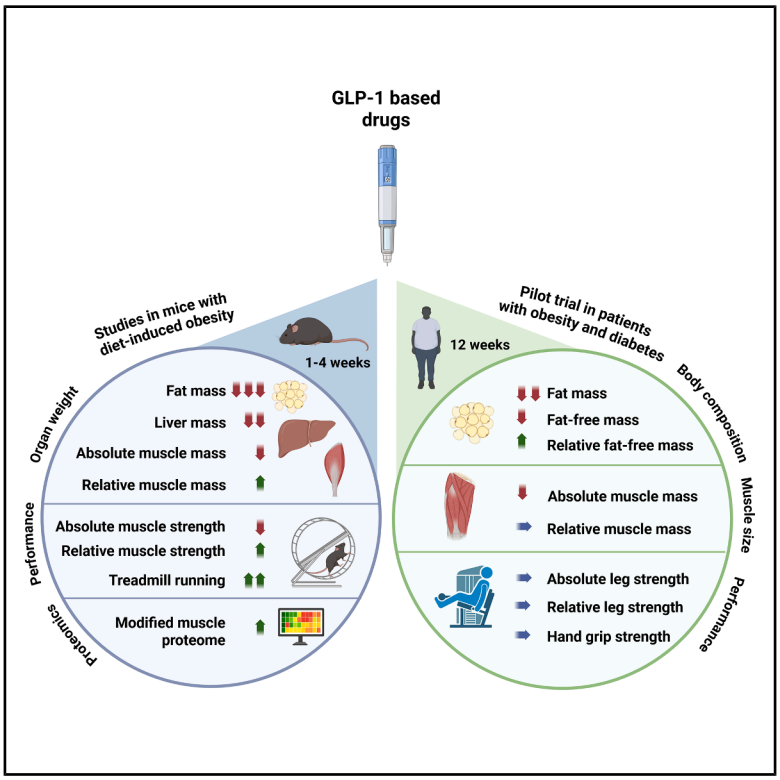
Weight loss with GLP-1 medicines does not result in a disproportionate loss of muscle mass or function in obese mice and humans

Graphical abstract



Highlights

- GLP-1 medicines reduce fat and liver mass more rapidly than skeletal muscle (SKM)
- Running performance is maintained or improved in mice on GLP-1 medicine
- GLP-1 medicine modifies the SKM proteome despite similar weight loss as calorie restriction
- Patients on GLP-1 medicine slightly reduce SKM mass but largely maintain strength

Authors

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In brief

Langer et al. show that GLP-1 medicines mildly reduce absolute muscle mass but improve relative mass and running performance in mice. Despite similar changes to body weight, GLP-1RA affects the muscle proteome differently compared to calorie restriction. A human pilot trial shows that muscle strength is maintained during GLP-1RA treatment.

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Weight loss with GLP-1 medicines does not result in a disproportionate loss of muscle mass or function in obese mice and humans

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SUMMARY

The large decrease in body weight with glucagon-like peptide-1 (GLP-1) medicines raises concern about a loss of lean body mass (LBM) and skeletal muscle. In this work, we present four pre-clinical studies and a proof-of-concept clinical trial that address this issue. We report that in obese mice, GLP-1 medicines predominantly reduce body fat alongside a small but significant decrease in LBM. Among lean tissues, loss of liver mass exceeds change in muscle mass. While absolute muscle mass and strength decrease, relative muscle mass and strength improve, resulting in better running performance. Interestingly, while atrophy is similar during immobilization, GLP-1 medicines have a distinct effect on the muscle proteome compared to calorie restriction. Patients with obesity on GLP-1 medicines improve their body composition without negatively affecting strength. Overall, in middle-aged mice and men, GLP-1 medicines slightly decrease absolute muscle values but positively impact body composition and mobility.
The clinical trial is registered on clinicaltrials.gov (NCT05606471).

INTRODUCTION

Globally, over 1 billion people suffer from obesity.[1] Incretin-based drugs offer a powerful treatment option for patients with obesity (PWO). Over the course of 68–72 weeks, once-weekly injections of semaglutide, a glucagon-like peptide-1 receptor agonist (GLP-1RA), and tirzepatide, a dual agonist for GLP-1 and glucose-dependent insulinotropic polypeptide (GIP), resulted in 12%–19% reductions in body weight (BW) compared to placebo, respectively.[2,3] However, both studies found that a significant portion of the weight loss was achieved through reductions in lean body mass (LBM). For example, roughly 40% of the weight loss in the Wilding study stemmed from LBM.[2] Similar values were very recently reported for the triple agonist (GLP-1R, GIPR, and glucagon receptor [GCGR]) retatrutide.[4] In contrast, LBM is commonly thought to contribute only ~25% during physiological weight loss, coined the "quarter fat-free mass (FFM) rule."[5] As such, the concern of a disproportionate loss of LBM, muscle mass, and muscle function with incretin-based drugs has spurred tremendous investments by the pharmaceutical industry[6] and the publication of review articles.[7,8] Despite the great interest in the matter, primary data remain sparse.

Importantly, LBM is not just comprised of skeletal muscle but also includes the heart, liver, bones, and other tissues.

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Additionally, fat mass has a lean component too.[8] As such, a decrease in LBM does not exclusively reflect changes in muscle mass but a diverse number of tissues including fat. Thus, distinguishing the effect of anti-obesity medications (AOMs) on muscle and other lean tissues in the absence of a direct measurement of muscle mass is challenging. Unfortunately, few clinical trials have directly assessed muscle after pharmacological weight loss.[9] No published clinical trial has directly measured changes in muscle function with AOMs. Even on the pre-clinical level, very few studies have reported changes to body composition alongside muscle mass and function for a drug that is approved for obesity.[10–12] Additionally, no study yet has explored how incretin-based poly-agonists affect skeletal muscle.

Here, we conducted a comprehensive assessment of changes to muscle mass and function with multiple incretin-based drugs and dosages in DIO mice. In the first study, we investigated the effect of a dual agonist (GLP-1R/GIPR) on changes in LBM, fat mass, and organ weight. In the second study, we examined the effect of GLP-1RA on muscle strength and endurance *in vivo*. In the third study, we explored whether in a known model of muscle wasting (i.e., immobilization), incretin-based therapies would accelerate muscle loss in obese mice. In a fourth study, we asked whether GLP-1RA and calorie restriction, with and without immobilization, would result in distinct changes to the skeletal muscle proteome. Finally, we conducted a proof-of-concept clinical study in PWO, where we tested changes in BW, LBM, muscle mass, and muscle strength before and after treatment with GLP-1RA.

RESULTS

A previous study of our group has shown that GLP-1RA treatment in mice predominantly reduces adipose tissue with only modest effects on muscle mass.[10] However, GLP-1R/GIPR dual agonists have proven more efficacious than GLP-1RA monotherapy in reducing BW in mice[13] and PWO[14] but no preclinical data on muscle mass are available. To address this gap, we treated DIO mice via daily subcutaneous (s.c.) injections with tirzepatide (50 μg/kg or ∼10 nmol/kg) or a vehicle for 14 days. We found a robust reduction in BW of ∼35% from baseline (Figures 1A and 1B), which was accompanied by a 73% reduction in body fat (Fig. 1C-D) and a 13% reduction in LBM (Figures 1E and 1F). The reduction in LBM contributed ∼20% to the total weight loss. We collected five muscles of the lower limbs, out of which only two showed a significant reduction in mass (Figure 1G), each in the range of ∼10% (Figure 1H). Importantly, since the loss of BW outpaced the decrease in muscle mass, the muscle weight per BW ratio (i.e., relative muscle mass) significantly improved in the extensor digitorum longus (EDL), soleus (SOL), and gastrocnemius (GSTN) and trended toward an improvement in the tibialis anterior (TA) and the quadriceps (QUAD) (Figure 1I). In contrast, all white adipose tissue depots (WAT) were significantly reduced by ∼50%–70%, with brown adipose tissue trending toward a significant reduction of ∼40% (Figures 1J and 1K). Interestingly, liver mass numerically decreased by ∼20% (Figure 1L). This decrease in liver mass is supported by results from a similar experiment, where we compared the effect of a GLP-1RA (semaglutide, 10 nmol/kg) and a GLP-1R/GIPR dual agonist (MAR709, 10nmol/kg) on BW, food intake, and body composition. Here, we found a decrease in BW of 28% and 33% for semaglutide and MAR709, respectively, which was associated with lowered food intake, a reduction in body fat of ∼51% and 62%, a decline in LBM of ∼5% and 8%, and a significant decrease in liver mass of ∼20% (Figures S1A–S1E). Overall, this suggests that while LBM loss with incretin-based drugs can reach significance in DIO mice, the weight loss is primarily driven by a decrease in fat mass, resulting in an improvement in the muscle mass-to-BW ratio. The more robust weight loss due to GLP-1R/GIPR dual agonism did not result in exacerbated muscle loss compared to our previous studies with GLP-1RA monotherapy.

To test whether these results translate to improved mobility and performance in mice, our next experiment focused on skeletal muscle function. We hypothesized that the observed increases in relative muscle mass in study 1 would translate to improved mobility and running performance in mice. DIO mice received either semaglutide (40 μg/kg, equivalent to ∼10 nmol/kg) (Sema) or a vehicle injection for 28 days. To provide important context for the changes to muscle with pharmacological weight loss, we compared our obese groups to lean control mice on a regular chow diet. Absolute BW of the DIO vehicle group further increased over the course of the study, while the Sema group decreased and the lean group remained mostly weight stable (Figure 2A). At 14 days, the difference between DIO and Sema was significant (Figure 2A). Relative to the DIO vehicle group, Sema treatment resulted in a 22% lower BW on day 28 (Figure 2B). In line with that, fat mass in the Sema group was significantly reduced compared to DIO starting at 14 days, with the difference on day 28 reaching 46% (Figure 2C). This reflects the fact that absolute fat mass continued to increase in the DIO group over the course of 28 days, while it was reduced in the Sema group (Figure 2D). In contrast, LBM was not statistically different between any of the groups at any time point (Figure 2E). There was, however, a significant decrease in LBM in the Sema group from day 0 to day 28 (Figure 2F). While the LBM decrease of 4% is much less substantial than the 46% decrease in fat mass, it still contributed roughly 32% of the total weight loss, owing to the fact that the mice carried almost twice as much LBM as fat at baseline (Figures 2D and 2F). This supports the notion that incretin-based therapies do result in significant loss of LBM in obese mice.

Since LBM is not exclusively comprised of skeletal muscle, we next investigated the major muscles of the lower limb. Interestingly, despite the decrease in LBM, we found only trends toward a reduction in absolute muscle mass with Sema (Figure 2G). However, the amount of muscle relative to the BW of the animals improved significantly in the SOL, TA, and GSTN with Sema compared to the DIO group (Figure 2H). In accordance with the first experiment, this suggests that while overall muscle mass tended to decrease with GLP-1RA, muscle mass was spared relative to overall BW loss. Since fat mass was lost to a larger degree than LBM, we measured inguinal WAT (iWAT) and epididymal WAT (eWAT) to confirm this. Indeed, iWAT and eWAT decreased robustly by ∼45% and ∼41% in the Sema

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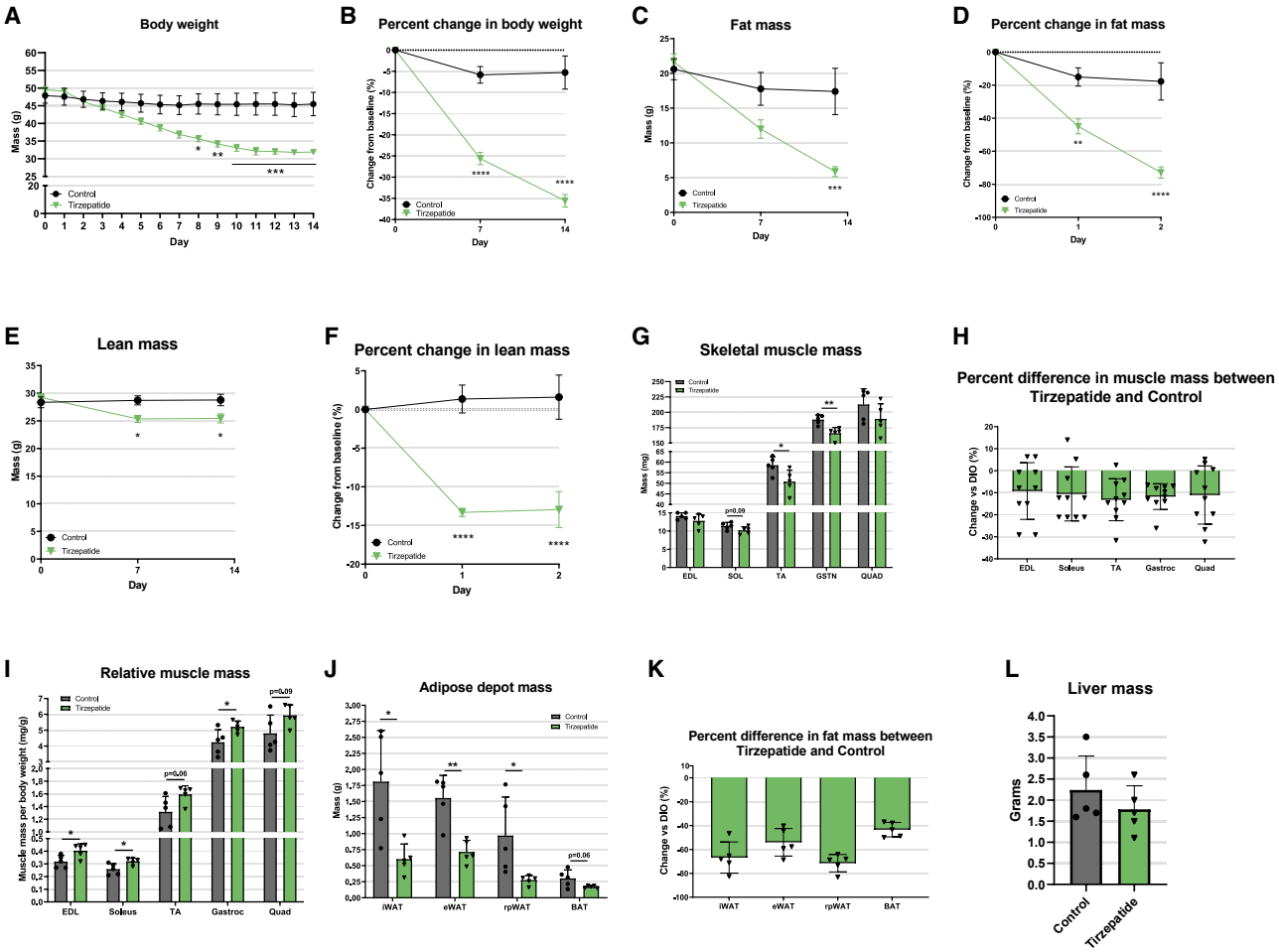

Figure 1. GLP-1R/GIPR dual agonism in DIO mice primarily reduces fat mass, and while LBM decreases significantly, the muscle mass-to-BW ratio improves

Body weight (A and B), fat mass (C and D), and LBM (E and F) development over the course of the 14-day intervention. Group differences were determined via a two-way ANOVA and Sidak's multiple comparison test. *, **, ***, and **** correspond to $p < 0.05$, $p < 0.01$, $p < 0.001$, and $p < 0.0001$ when comparing tirzepatide to the control group. Absolute muscle mass (G and H) and relative muscle mass (I) as well as adipose tissue depots (J and K) and liver mass (L) at the end of the intervention. Group differences were determined via unpaired *t* test between tirzepatide and the control group, with * and ** corresponding to $p < 0.05$ and $p < 0.01$, respectively. $n = 5$ per group, unless otherwise denoted. For the muscle mass data in (G), the average of both legs was calculated, while in (H) all individual values of the left and right leg are depicted together. Data are presented as individual data points for tissue weights or as mean ± SEM for data that consist of multiple time points.

compared to the DIO group (Figure 2I), resulting in a significantly lower adipose tissue-to-BW ratio for Sema (Figure 2J).

To better understand how these changes in body composition and tissue weights affect muscle function and mobility, we examined grip strength, running performance, and *in vivo* muscle contractility. Similar to the trends in muscle mass, absolute grip strength decreased slightly with Sema compared to DIO (Figure 2K), while relative grip strength improved (Figure 2L). This improvement in the ratio of muscle mass and function to BW also resulted in a robust increase in time to exhaustion for the Sema mice during a VO2 max treadmill test (Figure 2M). Indeed, the time to exhaustion and the total distance covered by the Sema mice were not just higher than the DIO mice but almost even with the lean control group (Figure 2N). Maximal torque during a force frequency test of the plantar flexors followed a similar trend: absolute force at higher frequencies was decreased in Sema versus (vs.) DIO mice (Figure 2O) but relative torque (i.e., the torque-to-BW ratio) was unchanged (Figure 2P). This is in line with similar, very recent reports.[12] Since we found absolute strength parameters (grip strength and force frequency) to be slightly reduced but endurance readouts improved (treadmill performance), we next tested how fatigue is affected during a repeated tetanus-torque test. In alignment with the previous results, the initial peak torque was significantly higher in the DIO compared to the Sema group (Figure 2Q). However, the decline in torque began significantly later in the Sema group, indicating an improvement in fatigue resistance (Figure 2R). This

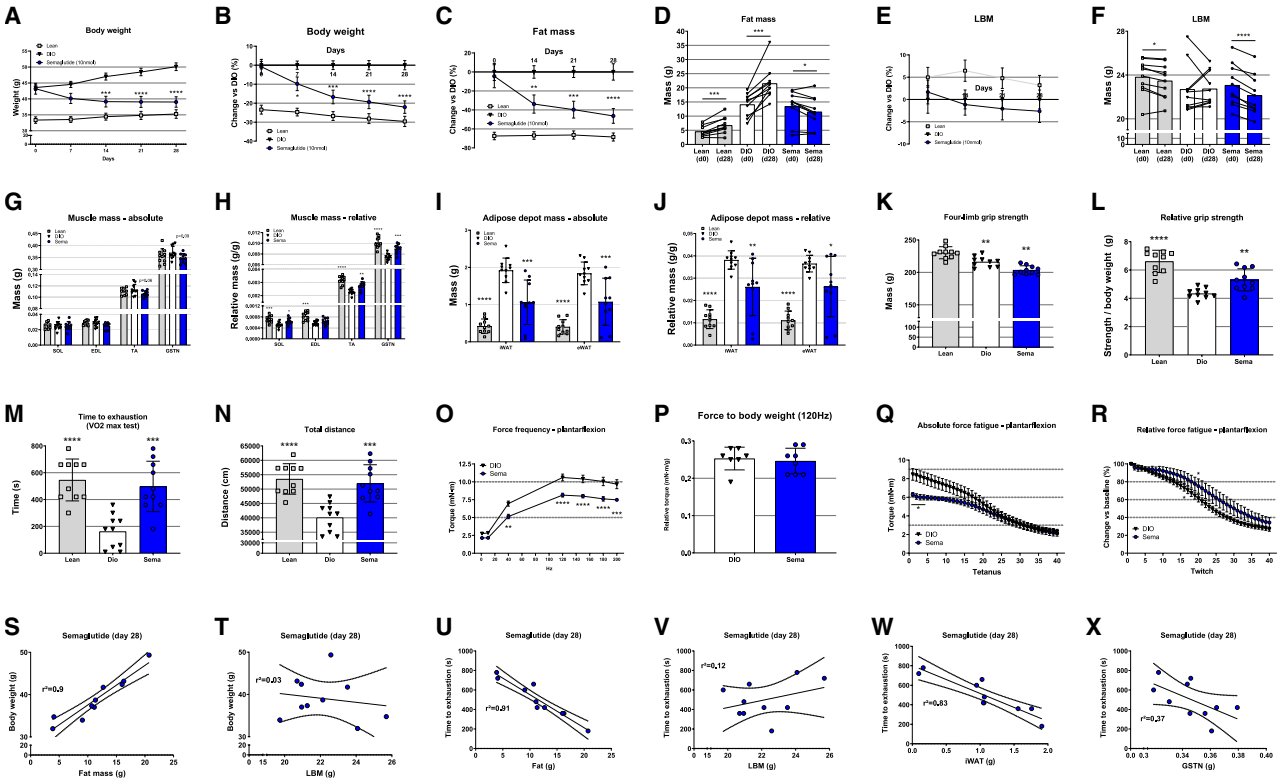

Figure 2. GLP-1RA reduces absolute muscle parameters but improves the muscle-to-BW ratio, leading to increased treadmill running performance

Absolute BW (A) and percent-change in BW vs. DIO control (B) over the course of the 28-day intervention. Group differences were determined via two-way ANOVA and Tukey's multiple comparison test. *** and **** denote $p < 0.001$ and $p < 0.0001$, respectively, for the comparison of Sema vs. DIO. Percent-change in fat mass vs. DIO control (C) and absolute values in fat mass at day 0 and day 28 of the intervention (D). Group differences were determined via two-way ANOVA and Tukey's multiple comparison test (C) or a paired *t* test (D). **, ***, and **** denote $p < 0.01$, $p < 0.001$ and $p < 0.0001$ for the comparison of Sema vs. DIO (C), while the asterisks in (D) denote the *p* values for the before-and-after comparison within each group. (E) and (F) show the equivalent for LBM. (G) and (H) display absolute and relative muscle mass, while (I) and (J) display absolute and relative adipose tissue mass. Muscles were collected from both legs and adipose tissue from all respective sites, with the sum being displayed. Group differences were determined via one-way ANOVA and Dunnett's multiple comparison test. *, **, ***, and **** denote $p < 0.05$, $p < 0.01$, $p < 0.001$, and $p < 0.0001$, respectively, compared to DIO. Absolute grip strength (K) and grip strength relative to body weight (L) from all four limbs were collected. Time to exhaustion during a treadmill VO2 max test (M) and total distance during the entirety of the running protocol (N) were recorded. For (K–N), group differences were determined the same way as (G–J). Force frequency (O), force-to-body weight ratio (P), absolute force fatigue (Q), and force fatigue relative to baseline (R) of the plantar flexors were assessed via *in vivo* contractility. Group differences were determined via two-way ANOVA and Sidak's multiple comparison test (O, Q, and R) or an unpaired *t* test (P). *, **, ***, and **** denote $p < 0.01$, $p < 0.001$, and $p < 0.0001$ for the comparison of Sema vs. DIO. Linear regression analysis was performed for BW vs. fat mass (S), BW vs. LBM (T), time to exhaustion vs. fat mass (U), time to exhaustion vs. LBM (V), and time to exhaustion vs. iWAT (W) and GSTN (X). All linear regression values were from day 28. Unless otherwise denoted, $n = 10$ per group for the first intervention (A–N and S–X), and $n = 7$–8 for the second intervention where *in vivo* contractility was tested (O–R). Data are presented as individual data points for tissue weights and running performance or as mean ± SEM for data that consist of multiple time points.

reinforces our finding that while absolute muscle mass and strength appear to mildly decrease with GLP-1RA, this effect is offset by a greater loss of adipose tissue, resulting in a higher relative muscle mass and increased mobility. To confirm that changes in fat mass and AT are more predictive for BW loss and running performance than LBM and muscle, we also conducted a sub-cohort linear regression analysis for these variables in the Sema group. We found that the final BW of the Sema mice on day 28 correlated remarkably with fat mass ($r^2 = 0.9$) (Figure 2S) but not with LBM ($r^2 = 0.03$) (Figure 2T). Similarly, time to exhaustion was negatively associated with fat mass ($r^2 = 0.91$) (Figure 2U) and iWAT ($r^2 = 0.83$) (Figure 2W) but not LBM ($r^2 = 0.12$) (Figure 2V). In fact, better running performance appeared mildly associated with smaller GSTN mass ($r^2 = 0.37$) (Figure 2X). This supported our hypothesis that increased relative muscle mass after AOM treatment would result in improved mobility and running performance in mice.

In our third study, we wanted to test how the use of incretin mimetics affects skeletal muscle during a known stimulus for wasting. Since most of our muscle-related results in DIO mice under ambulant conditions (i.e., study 1 and 2) could be explained through weight loss, we hypothesized that the more extreme combination of AOMs and immobilization may reveal more subtle, negative effects of incretin-based therapies. We chose immobilization, as it has been shown to result in rapid

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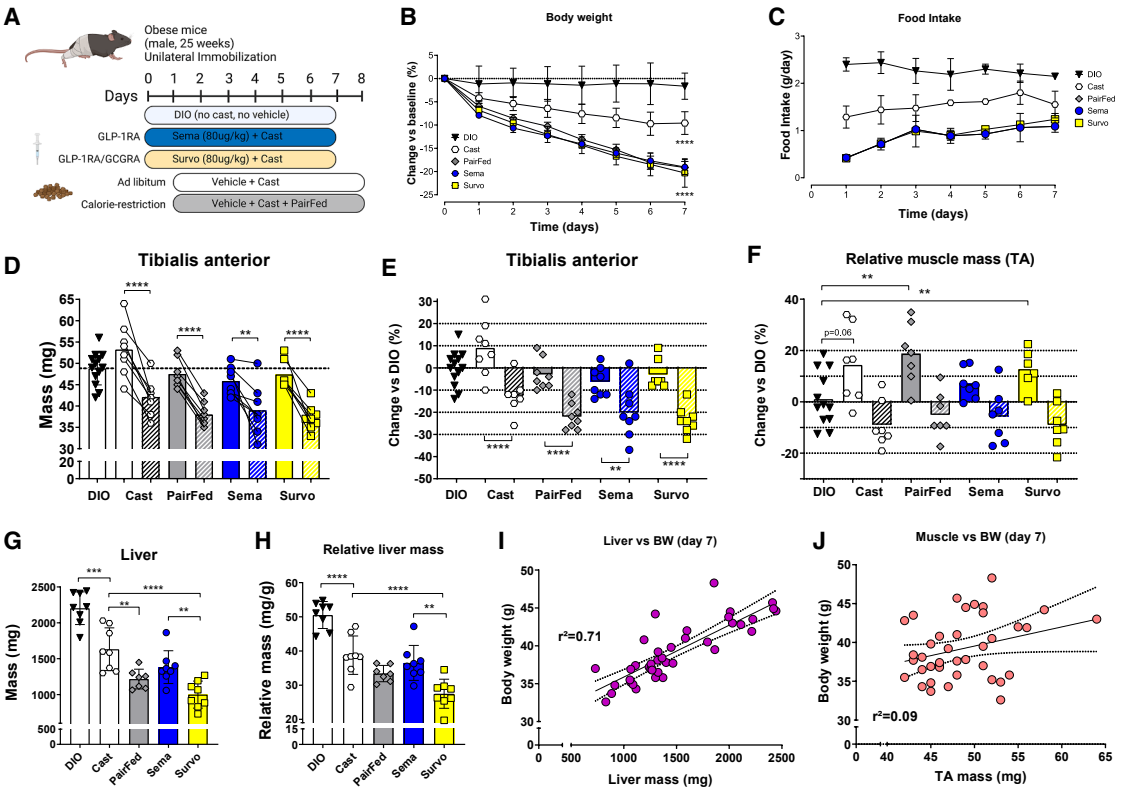

Figure 3. Pharmacological and physiological weight loss results in similar reductions of body weight and muscle mass during immobilization

Graphical representation of the immobilization experiment (A). Change in BW (B) and food intake (C) over the course of the 7-day intervention. Group differences for (B) were determined via two-way ANOVA and Tukey's multiple comparison test. Significant differences compared to the DIO control on day 7 are depicted as **** corresponding to $p < 0.0001$. Differences in food intake were not statistically assessed. Absolute TA mass (D), change in TA mass vs. baseline (E), and TA mass relative to the BW of the animals (F) were collected on day 7. Within-group differences between the control leg and the casted leg (D and E) were determined via a paired *t* test, with ** and **** denoting $p < 0.01$ and $p < 0.0001$, respectively. Across-group differences between the DIO group and all other interventions were determined via a one-way ANOVA and Dunnett's multiple comparison test (F), with ** denoting $p < 0.01$ when comparing the indicated leg to the DIO control. Absolute and relative liver mass (G and H) were collected on day 7. Group differences were determined via a one-way ANOVA and Tukey's multiple comparison test. **, ***, and **** denote $p < 0.01$, $p < 0.001$, and $p < 0.0001$ between the indicated groups, respectively. Linear regression analysis was performed for BW vs. liver mass (I) and BW vs. TA mass (J). All linear regression values were from day 7 of the intervention. $n = 8$ per group, unless otherwise denoted. For the muscle mass data of the DIO control group, left and right leg data were clustered as one group. Data are displayed as individual data points or mean ± SD.

and substantial disuse atrophy in rodents and humans.[15,16] Additionally, repeated periods of immobilization have been proposed as a major risk factor for sarcopenia.[17] We kept obese mice either untreated (DIO), unilaterally casted using a 3D-printed cast described by Moore and colleagues[18] and vehicle injected (Cast), GLP-1RA-treated (80 μg/kg, equivalent to ~20 nmol/kg semaglutide) and unilaterally casted (Sema), or GLP-1R/GCGR dual-agonist-treated (80 μg/kg, equivalent to ~20 nmol/kg survodutide) and unilaterally casted (Survo). We chose a GLP-1R/GCGR dual agonist in addition to GLP-1RA monotherapy, because pre-clinical data reported a significant reduction of LBM at higher doses,[19] and a phase 2a study found a reduction in circulating amino acids.[20] Based on glucagon's role in liver metabolism and its ability to suppress muscle protein synthesis,[21] this raises the concern that GCGRA could directly impact muscle loss by promoting the liberation of amino acids from skeletal muscle and conversion to glucose in the liver.[22,23] Additionally, data suggest that dual- and triple agonists containing a GCGRA are superior to GLP-1RA monotherapy for weight loss,[19,24] highlighting their future potential for clinical practice. To directly compare pharmacological with physiological weight loss, we also included a calorie-restricted group (PairFed) whose food intake was matched to the Sema group (Figure 3A).

After 7 days, the group with a cast and *ad libitum* feeding lost ~10% BW, while the PairFed, Sema, and Survo groups all lost ~20% BW (Figure 3B). That the cast control group lost significant weight despite *ad libitum* food access is in line with our previous observations in hindlimb-suspended rats.[15] The food intake over the course of the intervention mirrored the change in BW of all groups (Figure 3C). Casting decreased TA muscle mass in all treatment groups compared to the control leg (Figure 3D). Interestingly, compared to the untreated DIO group, the control leg of the casted mice with *ad libitum* food intake showed a numerical increase of ~9% (Figure 3E). This suggests that the ambulant leg was relied on and loaded more, resulting in mild compensatory hypertrophy. We did not observe a net increase in control TA mass in any other treatment groups, likely owing to the more robust weight loss. Indeed, when looking at

the amount of muscle mass relative to the BW, all casted groups showed a numerical increase in the control leg compared to untreated DIO mice (Figure 3F). The increase in the control leg of the Cast group showed a trend, and the improvements in the PairFed and the Survo group were even significant (Figure 3F). While the absolute decrease in TA mass of the casted leg ranged from −13% (Cast) to −24% (Survo) compared to untreated DIO mice (Figure 3E), the relative decrease (i.e., accounting for BW change) was only between −5% (PairFed) to −9% (Survo) (Figure 3F).

Since our data from Figure 1 suggested a robust involvement of the liver, we also investigated changes in liver mass after combined immobilization and incretin mimetic treatment. We found that liver mass decreased considerably in all treatment groups, −26% in Cast, −45% in PairFed, −37% in Sema, and −55% in Survo (Figure 3G). The fact that Survo achieved a significantly greater decrease in liver mass compared to Sema, despite similar weight loss, can likely be explained by the GCGRA effects on the liver and is in agreement with phase 2 clinical data that showed a robust reduction in liver fat of patients living with metabolic-dysfunction-associated steatohepatitis (MASH).[25] As such, a previously significant difference in liver mass between Cast and PairFed disappeared when we normalized to BW, but the difference between Sema and Survo persisted (Figure 3H). Further support for the strong involvement of the liver in regulating BW with calorie restriction and incretin-based therapies was found when we performed a linear regression analysis that included all groups in our study. While liver mass correlated tightly with BW (r^2 = 0.71) (Figure 3I), muscle mass in the uncasted TA was found to be a remarkably poor predictor of BW (r^2 = 0.09) (Figure 3J). Taken together, this suggests that when matched for calorie intake, pharmacological and physiological interventions result in comparable weight loss, with both having relatively modest effects on muscle mass in DIO mice. Consistent with this, immobilization caused a reduction in TA mass that was impacted by the extent of weight loss but not by the type of weight loss (i.e., physiological vs. pharmacological). Therefore, we could not confirm our hypothesis that immobilization exacerbates negative effects of AOMs on skeletal muscle mass of middle-aged DIO mice during weight loss.

To investigate whether the proteome differs in skeletal muscle after physiological and pharmacological weight loss, we performed unbiased proteomic profiling (LC-MS/MS) of the gastrocnemius and directly compared the semaglutide and pair-fed groups as depicted in Figure 3. For each group, we analyzed the control leg as well as the immobilized leg (Figure 4A). For both the untreated as well as the immobilized leg, we identified and quantified similar numbers of peptides and proteins (Figure 4B). Interestingly, despite the identical food intake (Figure 3C), the nearly identical change in body weight (Figure 3B) and a similar maintenance of muscle mass (Figure 3D), we found mild but robust differences between calorie restriction and GLP-1RA treatment. In the control leg, GLP-1RA induced a significant increase in diverse mitochondrial proteins such as SIRT5, COX5A, NDUFB8, or UQCRB compared to simple calorie restriction (Figures 4C–4F). The only proteins that decreased by at least 50% with GLP-1RA compared to calorie restriction were CMBL and CBR2 (Figure 4F). As a result, upregulated Gene Ontology (GO) terms for biological processes included mitochondrial respiratory chain complex I assembly and aerobic respiration, with corresponding upregulations in cellular compartments of not only the mitochondrion and mitochondrial inner membrane but also the muscle tendon junction and myofibrils (Figure 4C). Similarly, enrichment analysis of biological processes confirmed the strongest signals for increases in the aerobic electron transport chain and oxidative phosphorylation (Figure 4E). All proteins identified as "mitochondrial" in our study can be found in existing databases such as MitoCarta.[26] We identified a total of 307 mitochondrial proteins, which comprises 27% of MitoCarta. Twenty-eight proteins were significantly changed with semaglutide versus pair-feeding in the untreated control leg (26 up- and 2 down-regulated). In contrast, immobilization caused a very distinct pattern of changes in the muscles of semaglutide-treated compared to calorie-restricted animals. Here, the proteasome core complexes were the most enriched cellular components (Figures 4G–4I), with haptoglobin, LGALS3, PMSA5, DYNLL2, and HSPH1 being among the most prominently increased proteins with GLP-1RA treatment (Figure 4I). We also found a robust increase in MUSTN1, which is a protein that has been implicated in myogenesis and the regeneration of skeletal muscle after injury.[27] Interestingly, we also found a strong induction of CTSL (>17-fold) with GLP-1RA, albeit without reaching statistical significance. The only protein that was downregulated by at least 50% upon immobilization in the semaglutide versus the pair-feeding group was DNAJB5, a heat shock protein acting as a chaperone co-factor (Figure 4I). In a list of proteasome-related proteins from databases such as KEGG or the Reactome,[28,29] we identified 32 of 48 total proteins on the list (67%), of which 21 were significantly changed (all upregulated). Overall, these findings highlight that despite identical calorie intake, similar weight loss, and comparable changes in muscle mass, GLP-1RA results in significant molecular changes to the proteome in skeletal muscle compared to physiological weight loss, which is further modified by immobilization.

To explore how our findings in DIO mice translate to the human condition, we also performed a proof-of-concept clinical trial in patients with obesity and diabetes. Some endpoints of this trial such as body weight and body composition have been described previously.[30] However, here we report how GLP-1RA treatment affected muscle mass and function in our patients. Briefly, PWO and diabetes were escalated from 0.25 mg semaglutide s.c. weekly to 1 mg within the first 4 weeks of the trial (where tolerated) before maintaining this dose for another 8 weeks. BW, fat mass, and LBM all decreased significantly over the course of the 12 weeks.[30] However, the contribution of fat mass to weight loss was more than twice as high as LBM (70% fat to 30% LBM, respectively). This contribution of LBM is less than what the STEP 1 trial found for semaglutide[2] but slightly more than what the SURMOUNT-1 trial recently reported for tirzepatide.[31] Accordingly, we found that the fat-to-BW ratio significantly decreased in our patients (Figure 5A), while the LBM-to-BW ratio significantly increased (Figure 5B), resulting in favorable changes to body composition. In line with our animal data, absolute measures of muscle size decreased (Figure 5C), while relative muscle

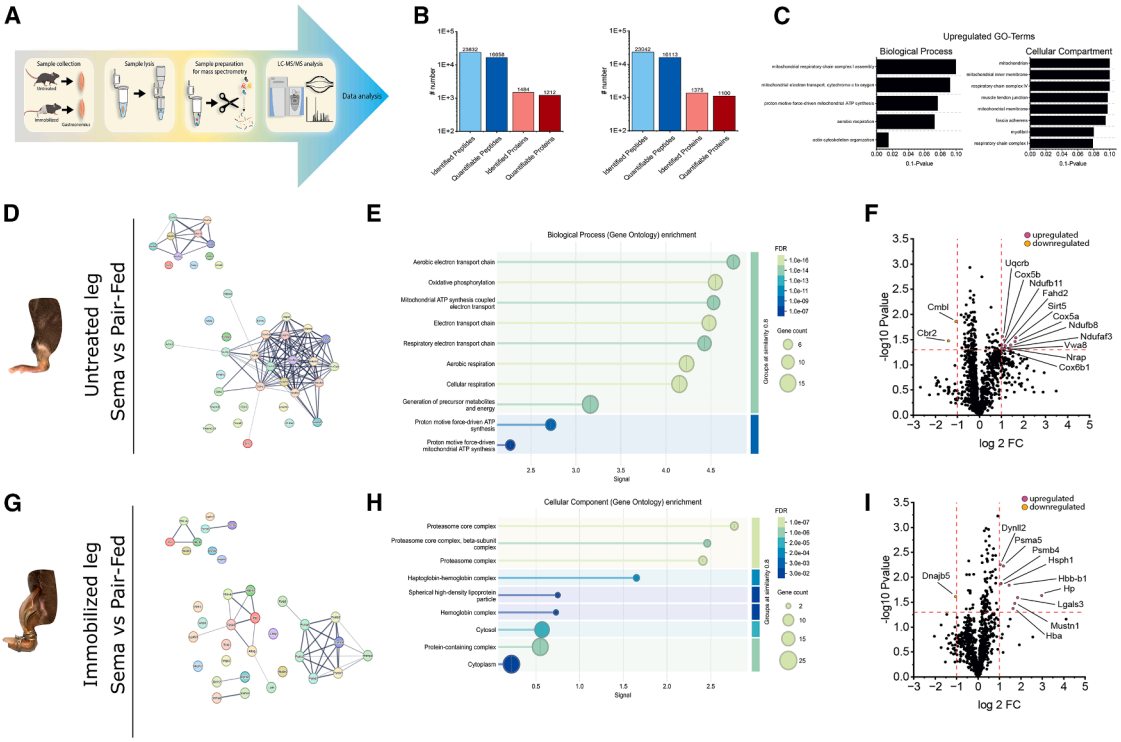

Figure 4. Pharmacological weight loss through GLP-1RA remodels the skeletal muscle proteome despite similar changes in body weight compared to pair-feeding

Graphical representation of the proteomics workflow (A). Number of identified peptides, quantified peptides, identified proteins, and quantified proteins for the "untreated" gastrocnemius muscle of the semaglutide and pair-feeding groups (left) and for the immobilized semaglutide and pair-feeding groups (right) (B). Upregulated biological processes and cellular compartments in muscle of untreated semaglutide versus pair-feeding mice (C). Signaling networks of highly regulated proteins in muscle of untreated semaglutide versus pair-feeding mice (D). Enrichment analysis of biological processes in untreated semaglutide muscle versus pair-feeding mice (E). Volcano plot of proteins in muscle of untreated semaglutide versus pair-feeding mice. Proteins that were increased to $\geq$2-fold or decreased to $\leq$0.5 are highlighted by name (F). Signaling networks of highly regulated proteins in muscle of immobilized semaglutide versus pair-feeding mice (G). Enrichment analysis of cellular components in immobilized semaglutide muscle versus pair-feeding mice (H). Volcano plot of proteins in muscle of immobilized semaglutide versus pair-feeding mice. Proteins that were increased to $\geq$2-fold or decreased to $\leq$0.5 are highlighted by name (I). Group differences in protein abundance were assessed via Student's *t* test, with results of $p < 0.05$ being deemed significant. $n = 4$ per group.

size was not negatively affected (Figure 5D). Interestingly, despite the significant reduction in cross-sectional area (CSA) of the vastus lateralis (VL), neither absolute muscle strength (maximum voluntary contraction [MVC] of the knee extensors) (Figure 5E) nor relative muscle strength decreased (Figure 5F). To our knowledge, direct measurements of muscle mass and strength have not yet been reported alongside each other for a clinical trial of incretin-based weight loss. The apparent discrepancy between muscle size and function could indicate that the reduction in CSA may not be entirely comprised of contractile proteins but also a decrease in other cellular components, like substrates. This would be in line with recent reports of decreased intramuscular fat after prolonged GLP-1RA/GIPRA treatment.[9] Similar to the lack of change in MVC, neither absolute (Figure 5G) nor relative hand grip strength (Figure 5H) was negatively affected by our intervention.

DISCUSSION

Body composition data from large-scale clinical trials have sparked a concern for muscle wasting with incretin-based therapies. Due to the widespread use of these drugs, such complications would have severe public health ramifications and thus have been the focus of various reviews.[7,8] Despite these growing concerns, primary data on muscle wasting with incretin treatments remain sparse, and no clinical study has yet assessed muscle mass and function in obese patients treated with weight loss drugs over a prolonged period of time. At the pre-clinical level, two studies have shown that myostatin/activin A inhibition can prevent muscle loss in pre-clinical models of incretin-treated obesity.[10,32] However, neither of the studies included a lean control or pair-fed group to explore whether the muscle mass changes with incretins were disproportionate or a simple function of weight loss. Similarly, a comprehensive assessment of muscle function and strength after AOM treatment has been missing, particularly in the clinical context.

We found support for the notion that muscle mass decreases as part of drug-induced weight loss. However, we did not find any evidence that this muscle loss was disproportionate or pathological. Across several pre-clinical scenarios and compounds, we found a consistent absolute decrease in muscle mass but a relative improvement in muscle-to-BW ratio. Even when

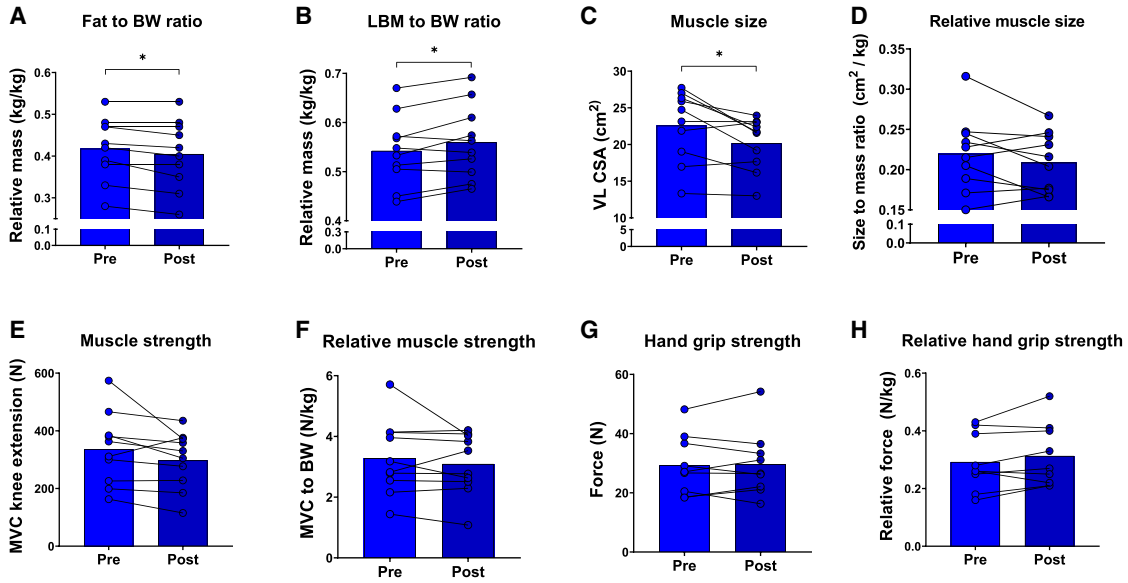

Figure 5. GLP-1RA treatment in patients results in a preferred reduction of body fat over LBM and a relative preservation of muscle strength despite a modest decrease in muscle size

Decrease in fat-to-BW ratio (A) and increase in LBM-to-BW ratio (B) after 12 weeks of GLP-1RA treatment. Absolute muscle size (C) and relative muscle size (D) as well as absolute muscle strength (E) and relative muscle strength (F), and absolute as well as relative hand grip strength (G and H). Group differences were assessed via paired *t* test, with * and **** denoting $p < 0.05$ and $p < 0.0001$, respectively. $n = 10$ per group, unless otherwise denoted.

challenged with a high dose of a GLP-1RA or a GLP-1R/GCGR dual agonist during immobilization, changes in muscle mass were remarkably similar to physiological weight loss through calorie restriction. While maintaining muscle mass as the largest depot of glucose in the body has multiple metabolic advantages to offer, preserving muscle function might be even more important clinically.[33] This is particularly true in the context of aging, since strength is lost faster than muscle mass.[34,35] However, we found that the decrease in muscle strength was similar to the trend in muscle mass, with both conserved relative to BW. This resulted in a favorable power-to-BW ratio in GLP-1RA-treated mice, which led to substantially improved treadmill performance that was comparable to lean animals.

We also conducted a proteomics study aiming to unveil the effects of GLP-1RA on skeletal muscle signaling after unilateral immobilization and in the contralateral control leg. Interestingly, GLP-1RA results in a robust increase in mitochondrial proteins in skeletal muscle of the control leg compared to calorie restriction alone. Recent studies indicated a possible effect of GLP-1RA on muscle mitochondria *in vivo*, but none of them have investigated whether these are a function of weight loss or GLP-1RA specifically.[11,36] In the immobilized leg, GLP-1RA induced an increase in pathways associated with the proteasome and muscle remodeling. These findings suggest that GLP-1RA influences muscle metabolism and the proteome independently of food intake, weight loss, and muscle mass levels. Importantly, skeletal muscle also does not appear to have a functional GLP-1R,[37,38] even though reported in some publications.[39–41] This means that these effects could be mediated via a currently unidentified signaling axis of indirect effects and tissue crosstalk.

In our GLP-1RA clinical trial, we observed similar tendencies. While LBM decreased significantly, a much larger portion of the weight loss was due to decreased fat mass. Even though muscle size decreased significantly, maximum knee extension or hand grip strength remained unchanged. While our trial was only designed as a proof-of-concept and does not have the necessary sample size and duration to provide conclusive evidence, it did directly assess muscle function in obese patients treated with incretin mimetics. However, recent large-scale clinical trials such as the STEP 9 in obese patients with osteoarthritis support the notion that the disproportionately larger loss of fat compared to LBM results in improved mobility and physical function.[42] Similar findings were reported by the SLIM LIVER study, where trends toward improved gait speed were found despite a decrease in psoas muscle volume.[43] Remarkably, this was found despite the patients suffering from a combination of human immunodeficiency virus (HIV) and MASH. Together with our data, these findings support the view that for PWO and related metabolic diseases, reducing BW and fat mass are paramount to improving health and mobility in young and middle-aged patients, even if it comes at the expense of a mild loss of LBM and muscle.

Importantly, our results also underscore that whole-body LBM, and muscle mass should not be conflated. While this important concept has been pointed out by colleagues in the context of AOMs,[8,44] the contribution of organs outside of skeletal muscle to LBM changes with incretin-based therapies is still poorly understood. By comparing changes in liver and muscle mass simultaneously, we found that the relative change in liver mass is much more pronounced during weight loss in obese mice. Importantly, we found this to be the case with various AOMs (GLP-1RA,

GLP-1RA/GIPRA, and GLP-1RA/GCGRA) as well as physiological weight loss (i.e., calorie restriction), with the effect being most pronounced for GLP-1RA/GCGRA. The fact that liver mass is so robustly affected by weight change raises another important issue for the interpretation of body composition data, highlighting that changes to LBM cannot be extrapolated to muscle mass or function. This is particularly important given that the tool routinely used to assess body composition in clinics, dual X-ray absorptiometry (DXA), cannot distinguish between LBM (or more accurately, soft lean tissue) and fat that resides within predominantly lean tissues. In other words, a decrease in intrahepatic or intra-muscular substrate content (i.e., triglycerides or glycogen) cannot be distinguished by DXA and might be quantified as a decrease in soft lean tissue. Since we found a strong decrease in liver mass in our pre-clinical cohorts, and clinical trials have shown a robust decrease in liver fat[25,45,46] and glycogen[47] with incretin-based therapies that cannot be accurately captured via DXA, this could be another reason why some clinical trials have found more LBM loss than anticipated. Notwithstanding these methodological limitations and confounders, it is also important to point out that outside of two studies,[2,4] the majority of clinical studies with incretin-based treatments did not find a disproportionate contribution of LBM to weight loss but were remarkably close to the "quarter FFM rule."[8] In keeping with this, the REDEFINE 1 recently reported an absolute weight loss of 25 kg after 68 weeks for co-treatment with cagrilintide and semaglutide, of which 8.3 kg was LBM and 16.7 kg was fat.[48] Despite larger weight loss than with semaglutide alone, LBM did only contribute 33% to the total weight loss. The semaglutide-only group lost 18.2 kg, of which 5.1 kg was LBM and 13.1 kg was fat, meaning LBM contributed only 28% to total weight loss. Importantly, participants in the cagrilintide + sema group reported an 85% improvement in the IWQOL-Lite-CT Physical Function Score and a 97% improvement in the SF-36 Physical Function Score. Interestingly, these improvements were even more pronounced for individuals who had poor physical function at baseline. This implies that in middle-aged PWO, weaker individuals appear to physically benefit the most from robust body weight reduction despite significant regression of total LBM. As such, current evidence provided by our study and the literature does not support the concern for an accelerated loss of muscle mass, function, or mobility with incretin-based treatments in middle-aged obese mice or patients.

Limitations of the study

Nevertheless, our study has several limitations. For example, the animal experiments were exclusively performed in male mice. This is due to the fact that in response to a high-fat diet, female wild-type mice develop obesity at a much slower rate and to a lesser extent than male mice.[49] Moreover, pair-feeding may not perfectly capture the complexity of calorie-restricted food intake, differences in meal distribution, and macronutrient preferences during physiological weight loss in humans. Mechanistically, our proteomics analysis in this study only compared the effects of semaglutide treatment and pair-feeding in immobilized muscles as well as the contralateral control leg. Future studies will need to validate that the differences in protein levels have functional consequences in mice and humans. Furthermore, even though introducing a direct assessment of muscle mass and muscle strength alongside changes in BW and LBM, our clinical trial did not have the necessary power to allow for firm conclusions. Large-scale clinicals trials with chronic treatment regimen will have to confirm our data in broader patient populations. Additionally, future work will have to assess muscle mass and function in incretin-treated patients who have additional comorbidities, particularly conditions known to cause muscle wasting (i.e., sarcopenia, cachexia or heart disease). Similarly, even in the absence of a pathological effect of incretins on muscle, improved maintenance of muscle mass during weight loss may still confer metabolic and functional advantages that result in higher resilience toward age-related diseases. Therefore, finding therapeutic solutions targeting muscle mass and function continues to be of high clinical relevance. Lastly, long-term adherence to incretin-based drugs is known to be poor, with over half of the patients discontinuing treatment within 1 year,[50] raising the concern of repeated weight loss and regain, which may result in deteriorating body composition.[51,52] Future studies will need to address whether AOMs exacerbate these effects and explore the role of skeletal muscle in preventing it.

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact, Henning Tim Langer (henning-tim.langer@charite.de).

Materials availability

This study did not generate new unique reagents.

Data and code availability

- The mass spectrometry proteomics data have been deposited to the ProteomeXchange Consortium via the PRIDE[53] partner repository with the dataset identifier PRIDE: PXD070915.
- This paper does not report original code.
- Any additional information required to reanalyze the data reported in this work paper is available from the lead contact upon request.

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AUTHOR CONTRIBUTIONS

Conceptualization, H.T.L. and K.B.; experiments, H.T.L., N.K.G., C.M.T.H., T.R., M.A., J.M., L.B., E.N., D.L., O.A., N.H., and A.H.; analysis, H.T.L., K.B.,

J.R., E.N., P.M.T., D.L., T.D.M., O.A., P.J.A., I.I., A.H., and A.R.; visualization, H.T.L., K.B., K.N., J.R., E.N., D.L., P.J.A., I.I., A.H., and A.R.; supervision, H.T.L., K.B., J.R., B.B., P.M.T., T.D.M., P.J.A., I.I., A.R., K.N., and U.M.W.; writing—original draft, H.T.L. and K.B.; writing—review and editing, all authors.

DECLARATION OF INTERESTS

H.T.L. was previously employed by Boehringer Ingelheim (2023–2024) and has worked as a consultant for Actimed Therapeutics, Almac Discovery, Alchemab Therapeutics, and Novartis. None of these engagements were related to this manuscript. PMT's research contributing to this manuscript was conducted at the University of Pennsylvania while serving as a faculty member (2017–2025). P.M.T. is currently an employee of Eli Lilly and Company; however, the research contributing to this manuscript, as well as the discussion and viewpoints expressed, are not affiliated with, nor endorsed by, Eli Lilly and Company. P.M.T. is acting on their own in the preparation and submission of this manuscript. K.B. is a co-founder of SinewUS, a tendon loading company, and has consulted for food companies such as PepsiCo, Ynsect, Advanced Muscle Technologies, GelTor, Evergrain, and Digestiva. .D.M. is a co-founder of Bluewater Biotech, holds stocks from Eli Lilly and Novo Nordisk, and received speaking fees from Novo Nordisk, Eli Lilly, Boehringer Ingelheim, Merck, AstraZeneca, Rhythm Pharmaceuticals, and Mercodia.

DECLARATION OF GENERATIVE AI AND AI-ASSISTED TECHNOLOGIES IN THE WRITING PROCESS

Artificial intelligence was not used in the writing of this article.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

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SUPPLEMENTAL INFORMATION

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Chemicals, peptides, and recombinant proteins		
Tirzepatide (Figure 1)	Peptide Sciences	N/A
MAR709 (Figure S1)	Novo Nordisk (Indianapolis, USA)	N/A
Semaglutide (Figure S1)	Novo Nordisk (Indianapolis, USA)	N/A
Semaglutide (Figure 2)	Peptide Sciences	N/A
Semaglutide (Figure 3)	Cayman Chemical	Item No. 29969
Survodutide (Figure 3)	Cayman Chemical	Item No. 40972
Deposited data		
Proteomics	ProteomeXchange Consortium (PRIDE)	PXD070915
Experimental models: Organisms/strains		
C57BL/6J	Jackson Laboratories	RRID:IMSR_JAX:000664
Software and algorithms		
Spectronaut	Biognosys	N/A
Prism Version 10	GraphPad Software Inc.	RRID:SCR_002798
Other		
High-fat diet	Research Diets	D12492

EXPERIMENTAL MODEL AND STUDY PARTICIPANT DETAILS

All animal studies were approved by their respective authorities as listed for each individual experiment in the sections below. The proof-of-concept clinical trial received ethical approval from local authorities (East of England, Essex Research Ethics Committee) and was registered on clinicaltrials.gov (NCT05606471).

For the four pre-clinical studies, male C57Bl/6J DIO mice (Jackson Laboratory, #380050) between 18 and 19 weeks of age (Figures 1, 2, 3, and 4) or 54 weeks of age (Figure S1) were used. For the proof-of-concept clinical trial, male and female patients aged between 18 and 75 years with type 2 diabetes (T2D) and a body mass index (BMI) between 27 and 50kg*m^{-2} were recruited.

METHOD DETAILS

Sub chronic DIO study with GLP-1RA/GIPRA dual agonist treatment

All mouse experiments in Figure 1 were reviewed and approved by the University of Pennsylvania IACUC in accordance with National Institutes of Health guidelines. Male, diet-induced obese mice (18 weeks of age) on a C57Bl/6J background were purchased from Jackson Laboratory (#380050) and maintained on a high fat diet (60 kcal% from fat; Research Diets D12492) for the duration of the experiment. Mice were group housed and had *ad libitum* access to food. Tirzepatide (Peptide Sciences) or an equal volume of vehicle control (0.9% saline) were administered s.c. at 50 μg/kg once daily for 14 days. Body weight was measured daily and body composition was assessed at days 0, 7, and 14 using an EchoMRI body composition analyzer (EchoMRI, Houston, TX). Mice were euthanized on day 14 and fat pads (eWAT, iWAT, rpWAT, BAT), hindlimb muscles (TA, SOL, EDL, GSTN, QUAD), and liver were collected and weighed.

For the sub chronic study mentioned in Figure S1, all experiments were performed in accordance with the animal protection law of the European Union after permission by the government of Upper Bavaria, Germany. 54-week-old C57BL6/J male mice were double-housed and fed *ad libitum* with a high-fat diet (58% fat, D12331, Research Diets, New Brunswick, USA). Fat and LBM were measured via nuclear magnetic resonance technology (EchoMRI, Houston, USA). Semaglutide and MAR709 were provided by Novo Nordisk (Indianapolis, USA). Animals were injected daily (s.c.) with the drugs or vehicle. On day 14, the mice were sacrificed and liver weight recorded.

Sub chronic DIO study that assessed muscle function

Adult male C57BL/6J mice at 18 weeks old were purchased from JAX (Bar Harbor, ME, USA) after being fed for 12 weeks with either 60% HFD (Research Diets# D12492i; DIO mice, n = 20) or normal chow diet (NC; SAFE A04i; NC mice, n = 10). After receiving the

animals, the preconditioned DIO mice and lean NC mice were acclimated to Biomeostasis' animal facility for two weeks, followed by one week of daily habitation to s.c. injections (vehicle). Throughout the study, mice were group housed (n = 2/cage) and allowed *ad libitum* access to water and HFD or NC, and maintained in Biomeostasis' animal facility at ambient temperature (22°C) and in a humidity (40–60%) controlled room on a 12-h light-dark cycle. Animal handling and procedures were conducted under the protocols and/or guidelines approved by the Institutional Animal Care and Use Committee (IACUC) and the local ethics committee (Marseille, APAFIS #49719). Mice received daily s.c. injections of vehicle (saline) or 40μg/kg semaglutide (Peptide Sciences, Lot# VIM982031106-3) for 4 weeks. On D0 (prior to the first dosing), D14, D21 and D28, body composition was measured using a TD-NMR minispec Analyzer (LF90II, Bruker, Germany).

Grip strength

On D28, the combined forelimb and hindlimb grip strength was measured on a grid assembly connected to a grip strength meter (BIOSEB, BIO-GS3). Briefly, the mouse is placed over the top of the grid so that both its front paws and hind paws can grip the grid. Torso was kept parallel to the grid and the mouse was pulled back steadily until the grip was released entirely from the grid. Upon release, maximal grip strength was automatically recorded by the acquisition software (BIO-CIS; BIOSEB, France). The procedure was repeated 3 times per animal with 5 min intervals between measurements. Grip strength was calculated as the mean of the 3 repeated measurements.

Treadmill running

On D21, each mouse was allowed to acclimate to a metabolic treadmill for 10 min at 10 m/min with no incline (0°) in order to familiarize the animals with the treadmill before the exercise test. On D28, each mouse ran on a one-lane metabolic treadmill (OxyletPro - Panlab LE 8708) connected to a gas analyzer (OxyletPro – Panlab LE405) for VO2 assessment. Each mouse was allowed to acclimate to the treadmill for 1h to obtain baseline values. The treadmill was then switched on for 30 min of light running at 10 m/min with no incline (0°). Subsequently, VO2 max was assessed during a progressive test of 2 min segments where either speed or inclination was increased: 15 m/min at 0°, 20 m/min at 0°, 20 m/min at 5°, 20 m/min at 10°, 20 m/min at 15°, 20 m/min at 20°, 20 m/min at 25°, 25 m/min at 25° and 30 m/min at 25°. The VO2 max test was stopped when a mouse remained on the shock pad for 10s. VO2 max was calculated as the mean VO2 during the last 3 min of the protocol and "time to exhaustion" as the completed time on the treadmill per mouse.

In vivo contractility

To avoid interference between the assessment of muscle mass, running performance and contractility, muscle force was tested on a separate set of animals that underwent the same drug intervention as described above but without the grip strength and treadmill running. No NC animals were included in this experiment. On D28, *in vivo* muscle strength of the right leg was tested using an Aurora Scientific System (1305A *in vivo/in situ/in vitro* muscle test system - 5N), similar to what has been described previously.[54,55] Briefly, mice were anesthetized (isofluorane/oxygen, 1 L/min) and the upper portion of the hindlimb was held in place, with the right paw fixed to the pedal of the servomotor system (301C, Aurora Scientific Inc., Aurora, Canada) such that only dorsiflexion and plantar flexion of the ankle were possible. Plantarflexion was stimulated through insertion of needle electrodes adjacent to the sciatic nerve). The following protocols were used: for the force frequency assay, a series of stimulations were performed at increasing frequencies (0.2 ms pulse, 500 ms train duration): 1, 10, 40, 120, 140, 160, 180 and 200 Hz. For the fatigue assay, a series of 40 stimulations at 120 Hz (tetanus stimulation frequency) were performed. On D28, the mice were fasted for 4h before being collected, and the weight of the GSTN, TA, EDL, SOL, iWAT and eWAT recorded.

Immobilization experiment

All immobilization experiments were approved by the University of California Davis Institutional Animal Care and Use Committee under Protocol #23367. Forty male Black 6 DIO mice (age 19 weeks) were obtained from Jackson Laboratories and housed 4 per cage with 12h light/dark cycles and *ad libitum* access to food and water. The right limbs of mice were immobilized as described by Moore and colleagues.[18] Briefly, 3D printed casts were placed over the right knee and ankle joint and held in place using 10lb wire. Both joints were maintained in a neutral position over the next seven days. Over the course of the study, none of the animals removed their casts. Starting at the time of immobilization, animals received subcutaneous injections of 80μg/kg or ∼20nmol/kg semaglutide (Sema), 80μg/kg or ∼20nmol/kg survodutide (Survo), or vehicle. A separate group of true control DIO mice were maintained without casting or injection.

Food was weighed daily, and total consumption was divided by the number of mice per cage to determine average food intake. The average food intake of the Sema group was then weighed out and provided to a separate group of one-day delayed casted, vehicle injected and single-housed mice (Pairfed). BW of all animals were tracked daily for the 7 days of study. At collection, the casts were removed and the tibialis anterior muscle removed, weighed, and frozen in liquid nitrogen. Livers were removed and weighed to estimate lean mass loss from other metabolic tissues.

Sample preparation for proteomics

Surgically removed gastrocnemius tissue was snap-frozen in liquid nitrogen and stored at −80°C. The total muscle was lysed in 200 μL of 50 mM Tris-HCl (pH 7.8) buffer, 5% SDS, and cOmplete ULTRA protease inhibitor (Roche) using the Bioruptor (Diagenode) for 10 min (30 s on, 30 s off, 10 cycles) at 4°C. To ensure complete lysis we conducted an additional sonication step using an ultrasonic probe (30s, 1s/1s, amplitude 40%) followed by centrifugation at 4°C and 20,000 g for 15 min. Protein concentration of the supernatant was determined by BCA assay according to the manufacturer's protocol. Disulfide bonds were reduced by addition of 10 mM TCEP at 37°C for 30 min, and free sulfhydryl bonds were alkylated with 15 mM IAA at room temperature (RT) in the dark for 30 min 100 μg protein of each sample was used for proteolysis using the S-Trap protocol (Protifi) and using a protein to trypsin ratio of 20:1. The incubation time for trypsin was changed to 2 h at 42°C. Proteolysis was stopped using formic acid to acidify the sample (pH < 3.0).

All proteolytic digests were checked for complete digestion after desalting by using monolithic column separation (PepSwift monolithic PS-DVB PL-CAP200-PM, Dionex) on an inert Ultimate 3000 HPLC (Dionex, Germering, Germany) by direct injection of 1 μg sample. A binary gradient (solvent A: 0.1% TFA, solvent B: 0.08% TFA, 84% ACN) ranging from 5 to 12% B in 5 min and then from 12 to 50% B in 15 min at a flow rate of 2.2 μL/min and at 60°C, was applied. UV traces were acquired at 214 nm.[56]

Proteomics analysis

All samples were analyzed using an UltiMate 3000 RSLC nano UHPLC coupled to a QExactive HF mass spectrometer with the total amount of peptide applied always being 1 μg. The samples were first transferred to a 75 μm × 2 cm, 100 Å, C18 pre column with a flow rate of 10 μL/min for 20 min followed by a separation on the 75 μm × 50 cm, 100 Å, C18 main column with a flow rate of 250 nL/min and a linear gradient consisting of solution A (99.9% water, 0.1% formic acid) and solution B (84% acetonitrile, 15.9% water, 0.1% formic acid) where the pure gradient length was 120 min (3–45% Solution B). The gradient was applied as follows: 3% B for 20 min, 3–35% for 120 min, followed by 3 wash steps each ranging to 95% buffer B for 3 min. After the last washing step, the instrument was allowed to equilibrate for 20 min. The acquisition of MS data was performed in DIA (data independent acquisition) mode. Each sample analyzed was mixed with an appropriate amount of iRT standard (Biognosys). Full MS scans were acquired from 300 to 1100 m/z at a resolution of 60,000 (Orbitrap) using the polysiloxane ion at 445.12002 m/z as lock mass. The automatic gain control (AGC) was set to 3E6 and the maximum injection time to 20 milliseconds. Full MS scans were followed by 23 DIA windows, each covering a range of 28 m/z with 1 m/z overlap, starting at 400 m/z, acquired at a resolution 30,000 (Orbitrap) with an AGC set to 3E6 and nCE of 27 (CID).

Data analysis of MS-Acquisitions

For the analysis of the samples acquired with nano-LC-MS/MS in DIA mode, the data was introduced to the Spectronaut software (Biognosys) and analyzed with a direct DIA-based search. As library the human proteome data was selected from UniProt (www.uniprot.org) containing 20,404 entries. Search and extraction settings were kept as standard (BGS Factory settings) Normalization was done by the software, using global normalization based on the median.

For reliable label-free quantification, only proteins with ≥2 unique peptides were considered for further analysis. Subsequently, the average normalized abundances (determined using Spectronaut) were calculated for each protein and used to determine the ratio between the different types of samples. Finally, to determine the significance of the abundance changes, a Student's *t* test was performed for each protein using MS Excel to calculate the corresponding P-values.

Network analysis

The obtained list of significantly regulated proteins was introduced into the STRING database[57] to visualize the network of dysregulated proteins. For this purpose, the significance value for the interactions in the STRING network was calculated by combining the categories co-expression, experimental, knowledge and text mining from the database. The interaction score was set to 0.4 (corresponding to an average to better interaction confidence). This score serves as an indicator of the confidence of a "true" interaction between proteins and can be set to the values 0.1 to 1. A score of 0.5 would mean that every second interaction could be false or false positive. The smaller this value is chosen, the more confident the indicated interactions are.

Pilot clinical trial

Participant recruitment, eligibility criteria and study design have been described in prior publication reporting changes to body weight, -composition, and metabolic effects.[30] Data presented here are unpublished data on muscle mass and function following semaglutide treatment in PWO and (T2D). Briefly, male and female patients aged between 18 and 75 years that had been diagnosed with T2D and a BMI between 27 and 50kg*m^{-2} were recruited into the study. Participants followed the study schedule previously described and therefore received once weekly subcutaneous semaglutide (Novo Nordisk) for 12 weeks, commenced at 0.25 mg and escalated every two weeks to 1 mg. This rapid dose escalation was performed to maximise exposure to the full dose of semaglutide and facilitate the fairest comparison between the interventions. Adverse event data were collected and if participants experienced significant side effects, dose escalation was delayed as would in usual clinical practice.

Each participant in this group was supplied with dietary advice focused on portion size reduction, avoidance of food with high-fat content, keeping well hydrated, and increasing awareness of satiety. This advice was provided in a written format along with the

dosing schedule of semaglutide. Participants were guided through administration of the first dose and then self-administered the remaining doses. They were instructed to document each dose they administered, to ensure they followed the study's dosing schedule.

Clinical assessment of muscle size and strength

VL muscle structure was examined using B-mode ultrasonography while participants were laid in the supine position. The belly of the VL was identified by measuring its entire length and noting the midpoint of this line. Muscle cross-sectional area (CSA) was determined along the mid-line using panoramic image rendering between the medial and lateral borders. Muscle thickness was also measured along the longitudinal axis of the muscle by aligning the ultrasound probe along the fascicle plane at the VL midpoint. At least three images of each parameter were saved for subsequent analysis which was performed using ImageJ (National Institute of Health) software. MVC was assessed in an isometric dynamometer, with the leg fixed in a 90° flexion. The participant was asked to contract this leg as hard as possible against a force transducer, which simultaneously measures the force generated and displays this on a connected monitor. This is in contrast to 1-repetition maximum (1RM) testing which involves repeated attempts at moving a selected weight which increases following each successful attempt. Handgrip strength was assessed using a digital handgrip dynamometer (Grip D 5401, Takei Scientific Instruments, Japan).

QUANTIFICATION AND STATISTICAL ANALYSIS

Statistical analyses were performed using GraphPad Prism software v.8 (La Jolla, USA). Data in the main figures is reported as individual data points ±SD except for data consisting of multiple time points, which is reported as mean ± SEM for visibility. Statistical tests were according to the research question and included unpaired- and paired t-tests, one-way and two-way ANOVA, together with post-hoc analyses via Dunnett's, Tukey's and Sidak's multiple comparison tests, respectively. For linear regression analyses, the line of best fit was calculated and displayed together with the 95% confidence intervals and the r^2 value for goodness of fit. Significance was determined as $p < 0.05$ or smaller, with asterisk * corresponding to $p < 0.05$, ** to $p < 0.01$, *** to $p < 0.001$ and **** to $p < 0.0001$. The underlying statistical test for each comparison that resulted in a significant difference is listed in the descriptions corresponding to the figure.