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Scriptaid enhances skeletal muscle insulin action and cardiac function in obese mice

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Aim: To determine the effect of Scriptaid, a compound that can replicate aspects of the exercise adaptive response through disruption of the class IIa histone deacetylase (HDAC) corepressor complex, on muscle insulin action in obesity.

Materials and methods: Diet-induced obese mice were administered Scriptaid (1 mg/kg) via daily intraperitoneal injection for 4 weeks. Whole-body and skeletal muscle metabolic phenotyping of mice was performed, in addition to echocardiography, to assess cardiac morphology and function.

Results: Scriptaid treatment had no effect on body weight or composition, but did increase energy expenditure, supported by increased lipid oxidation, while food intake was also increased. Scriptaid enhanced the expression of oxidative genes and proteins, increased fatty acid oxidation and reduced triglycerides and diacylglycerides in skeletal muscle. Furthermore, *ex vivo* insulin-stimulated glucose uptake by skeletal muscle was enhanced. Surprisingly, heart weight was reduced in Scriptaid-treated mice and was associated with enhanced expression of genes involved in oxidative metabolism in the heart. Scriptaid also improved indices of both diastolic and systolic cardiac function.

Conclusion: These data show that pharmacological targeting of the class IIa HDAC corepressor complex with Scriptaid could be used to enhance muscle insulin action and cardiac function in obesity.

KEYWORDS

animal pharmacology

1 | INTRODUCTION

Several aspects of skeletal metabolism are impaired in metabolic diseases, such as obesity and type 2 diabetes, including insulin action,[1] mitochondrial function[2] and substrate oxidation,[3] which have an

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impact on aspects of systemic metabolism.[1] First-line pharmacological interventions used to treat metabolic diseases have only limited direct effects on normalizing skeletal muscle metabolism.[4,5] Exercise is one intervention that enhances these aspects of muscle metabolism and has additional whole-body benefits, such as increasing energy expenditure and lipid utilization.[6] New pharmacological strategies are needed to enhance muscle metabolism in patient populations that cannot exercise or have low exercise adherence. It has been proposed that discovery of pharmacological interventions that replicate aspects of the adaptive response to exercise could be an important step in developing more efficacious treatments of metabolic diseases.[7,8]

We recently validated the class IIa histone deacetylases (HDACs) as drug targets to replicate aspects of the adaptive response to exercise. Furthermore, we identified the hydroxamic acid-based compound Scriptaid as a drug that induced exercise-like effects, not through inhibition of HDAC activity, but through disruption of the class IIa HDAC corepressor complex.[9] Class IIa HDACs are transcriptional repressors of myocyte enhancer factor 2 (MEF2) transcription factors.[10] Unlike class I HDACs, class IIa HDACs do not possess intrinsic HDAC activity because of a key amino acid substitution in their active site.[11] Instead, their repressive activity is related to the recruitment of a corepressor complex that contains N-Cor/SMRT and HDAC3.[12] We have previously observed that class IIa HDACs are exported from the nucleus in skeletal muscle during exercise, thereby disrupting the class IIa corepressor complex, which was associated with the expression of MEF2-dependent genes.[13,14] More recently, we showed that disruption of the class IIa HDAC corepressor complex, through either genetic means or via Scriptaid, relieved MEF2 repression, increased the expression of genes involved in oxidative metabolism and increased oxidative flux in skeletal muscle.[9] Furthermore, chronic Scriptaid treatment in lean mice increased exercise performance, increased whole-body energy expenditure and lipid oxidation and reduced blood glucose and lipids;[9] however, whether Scriptaid has any therapeutic benefit for metabolic diseases such as obesity and type 2 diabetes is unknown. The aim of the present study, therefore, was to examine the effect of Scriptaid on muscle insulin action in diet-induced obese mice. As exercise is also known to have positive effects on cardiac muscle function, a secondary aim was to examine the effect of Scriptaid on cardiac morphology and function.

2 | MATERIALS AND METHODS

2.1 | Animal studies

All experimental procedures were approved by the Deakin University Animal Welfare Committee, which is subject to the Australian Code for the Responsible Conduct of Research. Male C57Bl6 mice (7-8 weeks old) were obtained from the Animal Resource Centre (Western Australia) and were housed in the Metabolic Research Unit Animal Facility. All mice were housed in a temperature- (22°C) and humidity-controlled environment with a 12-hour light:12-hour dark cycle, with food and water provided *ad libitum* for the duration of the study. After a 2-week acclimation period, mice were fed a diet consisting of 43% of calories from fat (SF04-001 Rodent Diet; Specialty Feeds, Glen Forrest, Washington) for a 12-week period, after which mice were assigned to either vehicle or Scriptaid groups, such that each group had equivalent average body weight. Mice received single daily intraperitoneal (i.p.) injections of Scriptaid (1 mg/kg body weight; Santa Cruz Biotechnology, Dallas, Texas) or vehicle (5% DMSO in 1 × PBS) in the afternoon (n = 10 per group) for 4 weeks then were humanely killed. All mice were housed 2 per cage during the treatment period. In the final 2 weeks of treatment, mice underwent indirect calorimetry (Fusion Metabolic System; AccuScan Instruments, Columbus, Ohio), an i.p. glucose tolerance test (GTT; 2 g/kg lean mass, 6-hour fast) and an insulin tolerance test (ITT; 0.75U/kg, 6-hour fast). Mice were not administered the treatment regimen on the morning of these tests and all procedures were separated by at least 5 days. Mice were housed in the indirect calorimetry chambers for 25 hours and measures were obtained over the final 24 hours. Energy expenditure and substrate oxidation rates were determined using the equations reviewed by Ferrannini.[15] Blood glucose was determined throughout the GTT and ITT from a drop of blood obtained from the tail using an Accu-Check Performa hand-held glucometer. To determine plasma insulin throughout the GTT, an additional 30 μL of blood was obtained from the tail using a negative displacement pipette at baseline and 15, 30 and 60 minutes after glucose administration. Body composition by EchoMRI scan was measured on the day before the mice were killed. Mice were fasted for 4 hours before being humanely killed by cervical dislocation. The extensor digitorum longus (EDL) and tibialis anterior (TA) muscles were collected for *ex vivo* glucose uptake and fatty acid oxidation assays, respectively. Another cohort of mice was treated in the exact same manner, and after 3 weeks of treatment underwent echocardiography (Phillips HD15 diagnostic ultrasound system with 15 mHz linear-array transducer; Phillips Healthcare, Amsterdam, the Netherlands) under isoflurane anaesthesia to assess cardiac structure and function. All echocardiography procedures, analyses and equations were performed as previously described.[16] Five days later, mice were humanely killed and blood was drawn by cardiac puncture and collected into tubes containing heparin, followed by centrifugation at 4°C at 10 000 ***g*** for 10 minutes and plasma was collected. Tissues were collected, weighed, snap-frozen in liquid nitrogen and stored at −80°C until analysis of gene and protein expression and lipid levels, while a portion of the left ventricle was fixed for histological analysis.

2.2 | Plasma analyses

Plasma insulin throughout the i.p. GTT was assessed using a Mouse Insulin ELISA kit (ALPCO Diagnostics, Salem, New Hampshire). Lipids were extracted from ~10 μL of plasma obtained from cardiac puncture using 20 volumes of chloroform:methanol (2:1) in a single-phase extraction process, recovering all lipids in a single phase suitable for liquid chromatography–mass spectrometry analysis, as previously described.[17]

2.3 | Tissue analyses

Gene expression profiling was performed by extracting RNA from tissues using RNA columns and reverse transcribing RNA to cDNA, as previously described.[18] Real-time RT-PCR was performed using Sybr green detection, and log-transformed CT values were normalized to cDNA concentration, determined by Oligreen (Life

Technologies, Carlsbad, California), to determine relative gene expression levels. Amplification of mRNA was performed using primers that we have published previously.[16,19] To determine protein expression, tissues were homogenized, protein determined using the BCA assay and immunoblotting with primary antibodies for HSL, pS473 Akt, p-pan protein kinase C (PKC; Cell Signaling Technology, Danvers, Massachusetts) CPT-1b and tubulin (Sigma-Aldrich, St Louis, Missouri) was performed as previously described.[18] *Ex vivo* glucose uptake and fatty acid assays were performed as previously described,[20] in the EDL and TA muscles, respectively. Briefly, basal or insulin-stimulated (10 nM) 2-Deoxy-D-glucose uptake was assessed after incubation with 10 μM 2-Deoxy-D-glucose, 0.5 μCi/mL 2-Deoxy-D-[1-^{3}H]glucose and 0.2 μCi/mL D-[1-^{14}C]Mannitol for exactly 10 minutes. For fatty acid oxidation assays, the oxidation of 0.5 μCi/mL of [1-^{14}C]palmitate was determined over 90 minutes. Muscle and liver lipids were extracted from ~10 mg of tissue, and mass spectrometer determination of lipids was performed as previously described.[21] The size of cardiomyocytes from the left ventricle was determined from haematoxylin and eosin stained sections, as previously described.[16]

2.4 | Statistical analyses

All data are expressed as mean ± standard error of the mean (s.e.m.). Data normality was assessed using SPSS statistical software. For normally distributed data, differences between groups were assessed using a t-test, and 1-, 2- and 3-way analysis of variance, as appropriate using Minitab statistical software. Specific differences between groups were identified using Tukey *post hoc* tests. For non-normally distributed data, non-parametric tests were used, with SPSS statistical software. Differences were considered statistically significant for P values < .05.

3 | RESULTS

3.1 | Effect of Scriptaid on whole-body energy balance

Male C57BL6 mice were rendered obese through 12 weeks of high-fat feeding before administration of vehicle or Scriptaid for a period of 4 weeks via daily i.p. injection. Scriptaid had no effect on body mass (Figure 1A), lean mass (Figure S1A) or fat mass (Figure S1B). Consistent with our previous observations with this drug in lean mice, Scriptaid administration increased whole-body energy expenditure in the light phase (Figure 1B), which was associated with increased lipid oxidation (Figure 1C). There was no change in carbohydrate oxidation (Figure S1C) or voluntary activity levels (Figure S1D) in Scriptaid-treated mice. Similar to our findings in chow-fed mice, Scriptaid administration resulted in a small but significant increase in food intake (Figure 1D). These data show that, similarly to our previous findings in lean mice, treatment of obese mice with Scriptaid increased energy expenditure, supported by an increase in lipid oxidation, but did not alter body weight.

3.2 | Scriptaid enhanced insulin action, increased the expression of metabolic genes, increased fatty acid oxidation and reduced lipids in skeletal muscle

The effect of Scriptaid on skeletal muscle in obese mice was examined. Consistent with obesity-induced insulin resistance, insulin did not significantly increase *ex vivo* 2-deoxyglucose uptake by EDL muscles from vehicle-treated mice (Figure 2A). By contrast, insulin significantly increased 2-deoxyglucose uptake by EDL muscles from Scriptaid-treated mice (Figure 2A). Scriptaid did not increase GLUT4 or Syntaxin 4 protein levels (Figure S2A), suggesting mechanisms other than enhanced capacity of the glucose transport system were contributing to this effect. In an effort to explain this effect on skeletal muscle insulin action, we examined the effect of Scriptaid on exercise-like adaptive responses in these mice. Our previous studies have shown that Scriptaid increases a number of exercise-responsive genes involved in lipid metabolism in skeletal muscle.[9] Similarly, Scriptaid increased the expression of *Hsl*, *Pdk4*, *Cpt-1b*, *Sdhb*, *Atp5d* and *Pgc-1α* (Figure 2B). Scriptaid did not significantly increase HSL protein, but did increase the protein levels of CPT-1β (Figure 2C). *Ex vivo* fatty acid oxidation assays were performed in the TA muscle, and complete oxidation of palmitate to CO_2 was increased in Scriptaid-administered mice (Figure 2D), while acid soluble metabolites that are associated with incomplete fatty acid oxidation and insulin resistance[22] were reduced (Figure S2B). This was associated with a reduction in skeletal muscle triglycerides (TG; Figure 2E) and diacylglyceride (DG; Figure 2F). There was no change in muscle ceramide (Figure S2C). As certain DG species have been associated with insulin resistance,[23] these were assessed using a targeted lipidomics approach. A number of DG species incorporating 16 and 18 carbon fatty acids were reduced in Scriptaid-treated mice (Figure 2G). As DGs are thought to induce skeletal muscle insulin resistance via PKC activation that ultimately inhibits insulin signalling,[24] pan-PKC phosphorylation was assessed. There was no difference in PKC phosphorylation between vehicle- and Scriptaid-treated mice (Figure S2D). Furthermore, insulin-stimulated Akt phosphorylation was not different between vehicle- and Scriptaid-treated mice (Figure S2E), consistent with the idea that impairments in insulin signalling are not obligatory for insulin resistance.[25] Nonetheless, these data show that Scriptaid increases skeletal muscle insulin sensitivity, metabolic gene expression and fatty acid oxidation and reduces lipids in skeletal muscle of obese mice.

Although not a primary aim of the study, whole-body glucose homeostasis was next assessed in these mice. Scriptaid had no effect on glucose tolerance during an i.p. GTT (Figure S3A). Insulin levels throughout the i.p. GTT were not different between vehicle- and Scriptaid-treated mice (Figure S3B). Blood glucose levels during the ITT were also not different between treatment groups (Figure S3C). Unlike our previous observations in lean mice,[9] plasma lipids were not altered in obese mice treated with Scriptaid (Figure S3D). We also assessed the effects of Scriptaid on aspects of liver metabolism. Scriptaid treatment had no effect on liver weight (data not shown) or gluconeogenic and lipogenic gene expression (Figure S3E). Furthermore, Scriptaid had no effect on hepatic TGs (Figure S3F), DGs (Figure S3G) or ceramides (Figure S3H).

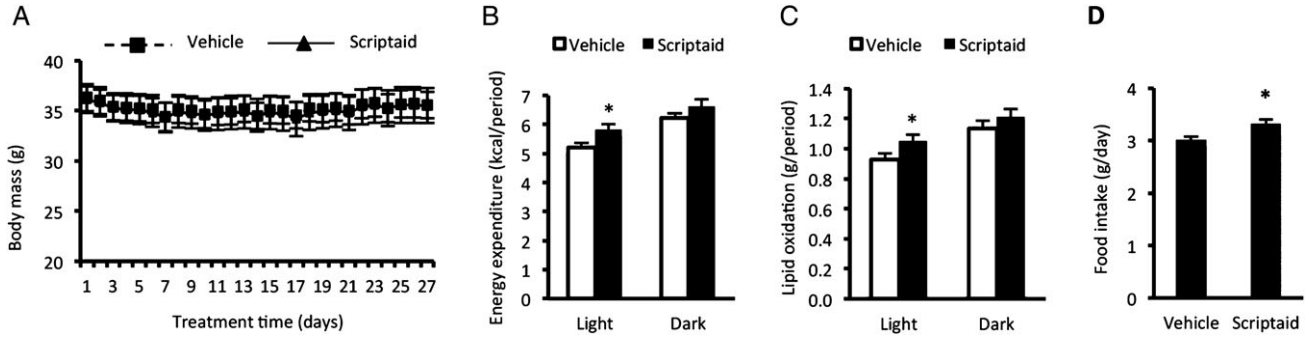

FIGURE 1 Effect of Scriptaid on whole-body energy balance. A, Body weight; B, energy expenditure; C, lipid oxidation and D, food intake in mice administered vehicle (5% DMSO in PBS) or Scriptaid (1 mg/kg body weight) via daily i.p. injection for 4 weeks (n = 10/group). Data represented as mean ± s.e.m. *Denotes significantly different from vehicle-treated mice ($P < .05$).

3.3 | Scriptaid reduces heart weight and increases cardiac expression of metabolic genes in obesity

Cardiomyopathy is common in obesity and is characterized by increased heart size and contractile dysfunction, which can occur independently of hypertension.[26] Indeed, cardiovascular disease is the major cause of morbidity and mortality in obesity. Many current drugs used to treat metabolic diseases have negligible effects on cardiovascular outcomes and obesity-induced heart failure in particular, which has led to calls for more in-depth evaluation of cardiovascular function in pre-clinical drug discovery and clinical trials for new metabolic disease drugs.[27] Because exercise is known to have positive effects on cardiac function and we have observed an increase in histone acetylation after acute Scriptaid administration,[9] we evaluated the effect of Scriptaid on obesity-induced cardiomyopathy. Scriptaid reduced absolute heart weight (Figure 3A), and the heart weight to tibia length ratio (Figure 3B), suggesting that the effect of Scriptaid was not attributable to altered body size. Indeed, Scriptaid reduced the diameter of cardiomyocytes (Figure S4A), assessed from heart sections stained with haematoxylin and eosin. The loss of the class IIa HDACs throughout development predisposes to cardiac hypertrophy through lost repression of foetal cardiac genes.[28] Gene expression analysis confirmed that Scriptaid did not reactivate components of the foetal gene programme, such as *ANF*, *BNP* and *β-MHC* (Figure S4B); however, as impaired energy metabolism is thought to be a primary driver of cardiac hypertrophy in obesity,[29] the effects of Scriptaid on the expression of genes involved in lipid metabolism in the heart was assessed. Similar to skeletal muscle, Scriptaid increased the expression of *Hsl*, *Cpt-1b*, Citrate synthase *(CS)*, *Atp5d* and *Pparα* in the heart (Figure 3C). These data show that Scriptaid reduces heart weight and enhances the expression of genes involved in lipid metabolism in the heart.

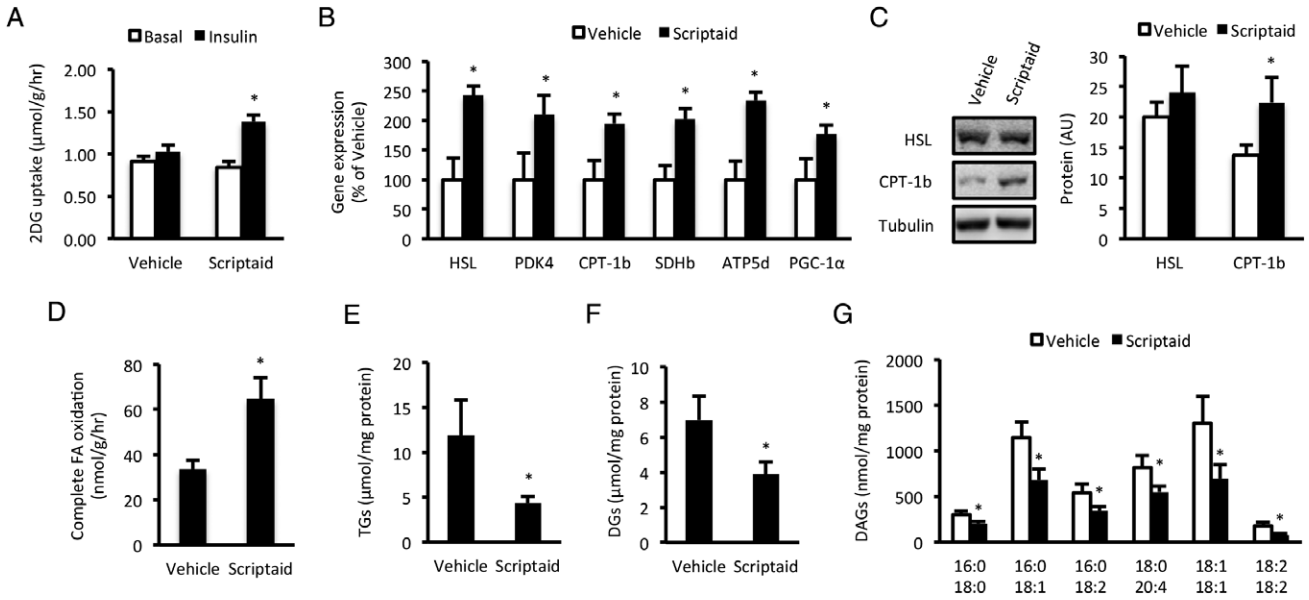

FIGURE 2 Scriptaid enhanced insulin action, and reduced lipids and mitochondrial reactive oxygen species production in skeletal muscle. A, 2-deoxyglucose (2DG) uptake in isolated EDL muscle incubated *ex vivo* with or without insulin (400 μU/mL); B, expression of metabolic genes; C, HSL and CPT-1b protein in EDL muscle; D, complete oxidation of palmitate to CO_2 in isolated TA muscle; E, TGs; F, DGs and G, DG subspecies in mice administered vehicle (5% DMSO in PBS) or Scriptaid (1 mg/kg body weight) via daily i.p. injection for 4 weeks (n = 8-10/group). Data represented as mean ± s.e.m. *Denotes significantly different from vehicle-treated mice ($P < .05$).

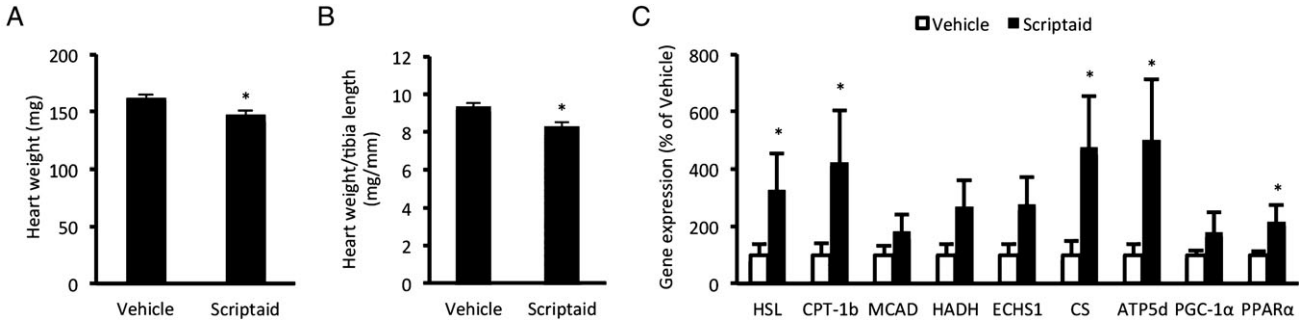

FIGURE 3 Scriptaid reduces heart weight and increases cardiac expression of metabolic genes in obesity. A, Heart weight; B, heart weight/tibia length and C, expression of metabolic genes in mice administered vehicle (5% DMSO in PBS) or Scriptaid (1 mg/kg body weight) via daily i.p. injection for 4 weeks (n = 8-10/group). Data represented as mean ± s.e.m. *Denotes significantly different from vehicle-treated mice ($P < .05$).

3.4 | Scriptaid enhances cardiac function and morphology in obesity

To determine whether the reduction in heart weight and increased expression of genes involved in lipid metabolism had any effect on cardiac function and morphology, another cohort of mice was examined by echocardiography. Specifically, echocardiography M-mode and Doppler flow analysis (Figure 4A) at both aortic and mitral valves on anaesthetized mice was performed. Scriptaid reduced the left ventricular internal diameter and increased the left ventricular posterior wall thickness and intraventricular septum thickness at the end of both diastole and systole and reduced estimated left ventricular mass (Table 1). These alterations are consistent with reduced pathological hypertrophy that included dilation of the left ventricle and impaired contractility. Indeed, Scriptaid reduced the estimated left ventricular mass (Table 1). Scriptaid administration enhanced the E:A ratio (Figure 4B), a key measure of diastolic function that represents the ratio between early and late filling of the atria. Scriptaid also enhanced measures of systolic function, including reduced ejection time (Figure 4C) and increased ejection fraction (Figure 4D) and fractional shortening (Figure 4E). These data show that Scriptaid enhances many indices of obesity-induced cardiomyopathy. This represents a further therapeutic benefit of class IIa HDAC corepressor disruption in metabolic disease states.

4 | DISCUSSION

Current drugs used to treat metabolic diseases have limited efficacy in directly normalizing skeletal muscle insulin sensitivity and cardiac function; however, exercise is an effective approach to enhance these aspects of metabolic disease pathology. Our recent finding that Scriptaid can recapitulate some aspects of the exercise adaptive response, through disruption of the class IIa HDAC

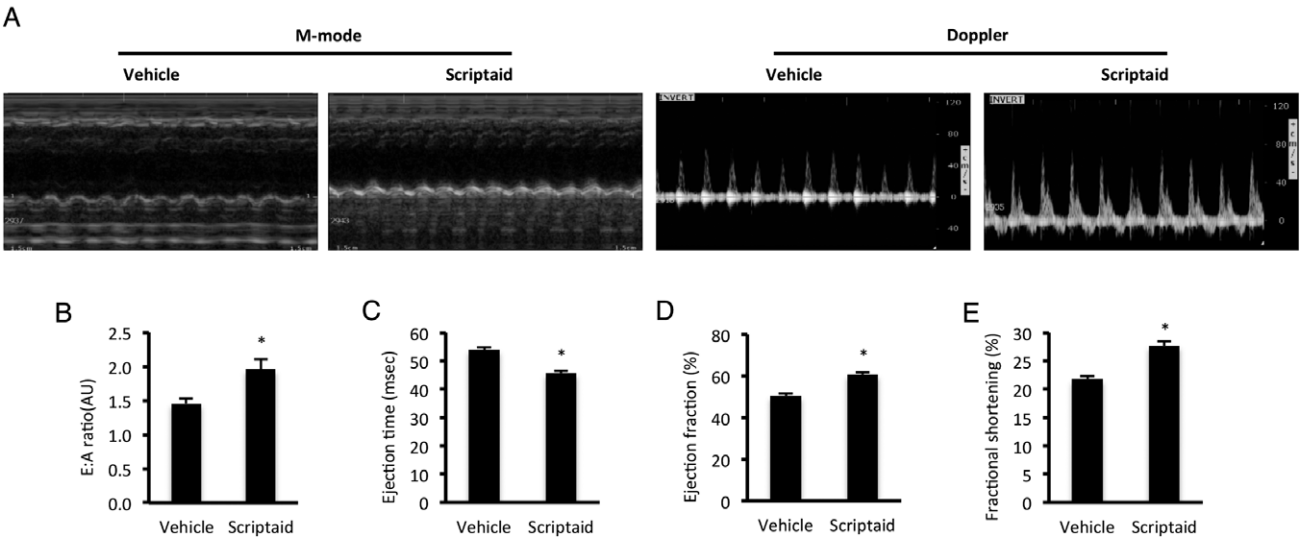

FIGURE 4 Scriptaid enhances cardiac function in obesity. A, Representative M-mode and Doppler ultrasound images; B, E:A ratio; C, ejection time; D, ejection fraction and E, fractional shortening in mice administered vehicle (5% DMSO in PBS) or Scriptaid (1 mg/kg body weight) via daily i.p. injection for 4 weeks (n = 8-10/group). Data represented as mean ± s.e.m. *Denotes significantly different from vehicle-treated mice ($P < .05$).

TABLE 1 Data from mice administered vehicle (5% DMSO in PBS) or Scriptaid (1 mg/kg body weight) via daily i.p. injection for 4 weeks (n = 8-10/group), showing that scriptaid enhances cardiac morphology in obesity

Variable	Vehicle	Scriptaid
Left ventricular internal diameter at diastole, mm	4.05 ± 0.19	3.53 ± 0.09*
Left ventricular internal diameter at systole, mm	3.17 ± 0.14	2.55 ± 0.08*
Left ventricular posterior wall thickness at diastole, mm	0.53 ± 0.01	0.59 ± 0.02*
Left ventricular posterior wall thickness at systole, mm	0.56 ± 0.01	0.66 ± 0.02*
Intraventricular septum thickness at diastole, mm	0.47 ± 0.01	0.54 ± 0.01*
Intraventricular septum thickness at systole, mm	0.52 ± 0.01	0.60 ± 0.02*
Estimated left ventricular mass, mg	114.6 ± 5.3	89.5 ± 4.3*

Data represented as mean ± s.e.m.

*Denotes significantly different from vehicle-treated mice ($P < .05$).

corepressor complex, prompted us to examine the efficacy of Scriptaid in obesity in the present study. Scriptaid administration to obese mice enhanced the expression of genes involved in oxidative metabolism, increased lipid oxidation, reduced lipid levels and enhanced insulin sensitivity in skeletal muscle. Scriptaid also reduced pathological cardiac remodelling and enhanced cardiac function. These findings suggest that targeted disruption of the class IIa HDAC corepressor complex could be used to enhance skeletal muscle insulin sensitivity and improve cardiac function in patients who cannot exercise. Furthermore, this therapeutic approach could be used in combination with existing therapies that target the liver, adipose tissue and the pancreas for a more holistic treatment of metabolic diseases.

Our previous studies showed that a major phenotypic effect of Scriptaid was to enhance skeletal muscle and whole-body lipid oxidation. In the present study in obese mice, we observed an increase in whole-body lipid oxidation and reduced levels of skeletal muscle TGs and DGs and more specifically, a number of DG species that have been associated with skeletal muscle insulin resistance. The putative mechanism by which DGs are thought to induce insulin resistance is through activation of PKC isoforms, which in turn serine phosphorylate and inactivate components of the proximal canonical insulin-signalling pathway. It appears clear, however, that the effects of Scriptaid on skeletal muscle insulin action were independent of this signalling mechanism, as pan-PKC phosphorylation and insulin-stimulated Akt phosphorylation were not different between treatment groups; however, enhanced skeletal muscle insulin action in Scriptaid-treated mice was also associated with reduced acid-soluble metabolites from incomplete fatty acid oxidation, which have been linked to skeletal muscle insulin resistance through as yet undefined mechanisms.[22] Identification of these mechanisms could also identify the exact mechanism by which Scriptaid enhanced skeletal muscle insulin action in obesity, but are likely to be linked with increased substrate turnover in skeletal muscle.

Another important observation from the present study was that Scriptaid was able to reduce cardiac hypertrophy and improve cardiac function in obese mice. These findings are significant as cardiovascular disease accounts for ~65% of deaths in obese and diabetic patients.[30] Current pharmacological approaches for metabolic diseases have limited or no efficacy against the development of heart disease.[31] Indeed, a limitation to a number of metabolic disease therapies has been adverse cardiovascular events[32]; therefore, the discovery of a therapeutic approach that not only enhances muscle metabolism, but also normalizes cardiac structure and function in obesity is a significant advance that could have a great impact on patient treatment and mortality. Ablation of class IIa HDAC family members throughout development has been associated with a predisposition for stress-induced cardiac hypertrophy, largely through re-activation of the foetal gene programme[33,34]; however, HDAC inhibitor use in adult stress models suggests that HDACs have divergent roles in hypertrophy in the developing and adult heart.[35,36] Reinforcing this view, Scriptaid had no effect on the expression of cardiac foetal genes such as ANF, BNP and β-MHC in the present study. Impaired energetics has been linked to the cardiomyopathy observed in obesity and type 2 diabetes[37] and Scriptaid enhanced the expression of a number of genes involved in fatty acid oxidation in the heart, just as it did in skeletal muscle. Additional studies will be required to determine whether Scriptaid enhances insulin action and reduces lipids in the heart.

Despite enhancing skeletal muscle insulin action, Scriptaid had no effect on glucose tolerance or whole-body insulin action in obese mice. These data are consistent with a number of studies in genetically modified animals, showing that alterations in skeletal muscle insulin action have limited impact on these tests. For example, muscle insulin receptor knockout mice have impaired muscle insulin action, but no defect in whole-body glucose or insulin tolerance.[38] Similarly, muscle-specific ablation of glycogen synthase impaired muscle insulin action, but enhanced whole-body glucose tolerance.[39] These data are consistent with the liver as being the primary tissue influencing glycaemia during these tolerance tests in mice.[40] Although we found that Scriptaid did not alter hepatic gluconeogenic and lipogenic gene expression or hepatic lipids, we cannot exclude the possibility that Scriptaid has deleterious effects on hepatic insulin action or glucose effectiveness. An area of future research will be to determine whether compounds that enhance muscle metabolism, such as Scriptaid, enhance the clinical efficacy of existing metabolic disease drugs when used in combination. A further application of this therapeutic approach could be to enhance exercise tolerance in patients who cannot or will not exercise, thereby allowing patients to benefit from the wide-ranging health benefits of exercise.

In conclusion, the present study reports that Scriptaid, which disrupts the class IIa corepressor complex in skeletal muscle, enhances muscle insulin action and cardiac function in obesity. As current therapeutic approaches have limited efficacy in normalizing these aspects of metabolic disease, targeting the class IIa corepressor complex could provide a more holistic approach to combating these diseases.

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Conflict of interest

The authors declare that there is no conflict of interest associated with this manuscript.

Author contributions

M. H. and S. L. M. conceived the research. V. G., T. C., K. R. W., M. H. and S. L. M. designed experiments. V. G., T. C., K. V., D. C., S. D. M., C. S., S. M., K. A. M., S. M. G. and R. V. E. performed experiments and analysed data. G. S., M. A. F., and G. R. S. provided technical expertise and reagents. V. G., T. C. and S. L. M. wrote the manuscript. All authors edited and approved the manuscript.

REFERENCES

1. DeFronzo RA, Tripathy D. Skeletal muscle insulin resistance is the primary defect in type 2 diabetes. *Diabetes Care*. 2009;32(suppl 2):S157-S163.
2. Petersen KF, Dufour S, Befroy D, Garcia R, Shulman GI. Impaired mitochondrial activity in the insulin-resistant offspring of patients with type 2 diabetes. *N Engl J Med*. 2004;350:664-671.
3. Kelley DE, Simoneau JA. Impaired free fatty acid utilization by skeletal muscle in non-insulin-dependent diabetes mellitus. *J Clin Invest*. 1994;94:2349-2356.
4. Stumvoll M, Nurjhan N, Perriello G, Dailey G, Gerich JE. Metabolic effects of metformin in non-insulin-dependent diabetes mellitus. *N Engl J Med*. 1995;333:550-554.
5. Arnes L, Gonzalez N, Tornero-Esteban P, et al. Characteristics of GLP-1 and exendins action upon glucose transport and metabolism in type 2 diabetic rat skeletal muscle. *Int J Mol Med*. 2008;22:127-132.
6. Sjodin AM, Forslund AH, Westerterp KR, Andersson AB, Forslund JM, Hambraeus LM. The influence of physical activity on BMR. *Med Sci Sports Exerc*. 1996;28:85-91.
7. Narkar VA, Downes M, Yu RT, et al. AMPK and PPARdelta agonists are exercise mimetics. *Cell*. 2008;134:405-415.
8. Carey AL, Kingwell BA. Novel pharmacological approaches to combat obesity and insulin resistance: targeting skeletal muscle with 'exercise mimetics'. *Diabetologia*. 2009;52:2015-2026.
9. Gaur V, Connor T, Sanigorski A, et al. Disruption of the class IIa HDAC corepressor complex increases energy expenditure and lipid oxidation. *Cell Rep*. 2016;16:2802-2810.
10. McKinsey TA, Zhang CL, Olson EN. Control of muscle development by dueling HATs and HDACs. *Curr Opin Genet Dev*. 2001;11:497-504.
11. Lahm A, Paolini C, Pallaoro M, et al. Unraveling the hidden catalytic activity of vertebrate class IIa histone deacetylases. *Proc Natl Acad Sci U S A*. 2007;104:17335-17340.
12. Fischle W, Dequiedt F, Hendzel MJ, et al. Enzymatic activity associated with class II HDACs is dependent on a multiprotein complex containing HDAC3 and SMRT/N-CoR. *Mol Cell*. 2002;9:45-57.
13. McGee SL, Fairlie E, Garnham AP, Hargreaves M. Exercise-induced histone modifications in human skeletal muscle. *J Physiol*. 2009;587:5951-5958.
14. McGee SL, Hargreaves M. Exercise and myocyte enhancer factor 2 regulation in human skeletal muscle. *Diabetes*. 2004;53:1208-1214.
15. Ferrannini E. The theoretical bases of indirect calorimetry: a review. *Metabolism*. 1988;37:287-301.
16. Venardos K, De Jong KA, Elkamie M, Connor T, McGee SL. The PKD inhibitor CID755673 enhances cardiac function in diabetic db/db Mice. *PLoS ONE*. 2015;10:e0120934.
17. Meikle PJ, Barlow CK, Mellett NA, et al. Postprandial plasma phospholipids in men are influenced by the source of dietary fat. *J Nutr*. 2015;145:2012-2018.
18. McGee SL, van Denderen BJ, Howlett KF, et al. AMP-activated protein kinase regulates GLUT4 transcription by phosphorylating histone deacetylase 5. *Diabetes*. 2008;57:860-867.
19. McGee SL, Swinton C, Morrison S, et al. Compensatory regulation of HDAC5 in muscle maintains metabolic adaptive responses and metabolism in response to energetic stress. *FASEB J*. 2014;28:3384-3395.
20. O'Neill HM, Lally JS, Galic S, et al. AMPK phosphorylation of ACC2 is required for skeletal muscle fatty acid oxidation and insulin sensitivity in mice. *Diabetologia*. 2014;57:1693-1702.
21. Matthews VB, Allen TL, Risis S, et al. Interleukin-6-deficient mice develop hepatic inflammation and systemic insulin resistance. *Diabetologia*. 2010;53:2431-2441.
22. Koves TR, Ussher JR, Noland RC, et al. Mitochondrial overload and incomplete fatty acid oxidation contribute to skeletal muscle insulin resistance. *Cell Metab*. 2008;7:45-56.
23. Bergman BC, Hunerdosse DM, Kerege A, Playdon MC, Perreault L. Localisation and composition of skeletal muscle diacylglycerol predicts insulin resistance in humans. *Diabetologia*. 2012;55:1140-1150.
24. Erion DM, Shulman GI. Diacylglycerol-mediated insulin resistance. *Nat Med*. 2010;16:400-402.
25. Hoehn KL, Hohnen-Behrens C, Cederberg A, et al. IRS1-independent defects define major nodes of insulin resistance. *Cell Metab*. 2008;7:421-433.
26. Isfort M, Stevens SC, Schaffer S, Jong CJ, Wold LE. Metabolic dysfunction in diabetic cardiomyopathy. *Heart Fail Rev*. 2014;19:35-48.
27. McMurray JJ, Gerstein HC, Holman RR, Pfeffer MA. Heart failure: a cardiovascular outcome in diabetes that can no longer be ignored. *Lancet Diabetes Endocrinol*. 2014;2:843-851.
28. Chang S, McKinsey TA, Zhang CL, Richardson JA, Hill JA, Olson EN. Histone deacetylases 5 and 9 govern responsiveness of the heart to a subset of stress signals and play redundant roles in heart development. *Mol Cell Biol*. 2004;24:8467-8476.
29. Lopaschuk GD, Folmes CD, Stanley WC. Cardiac energy metabolism in obesity. *Circ Res*. 2007;101:335-347.
30. Geiss LS, Herman WH, Smith PJ, National Diabetes Data Group. *Diabetes in America*. Bethesda, MD: National Institutes of Health, National Institute of Diabetes and Digestive and Kidney Diseases; 1995.
31. Forcheron F, Basset A, Abdallah P, Del Carmine P, Gadot N, Beylot M. Diabetic cardiomyopathy: effects of fenofibrate and metformin in an experimental model–the Zucker diabetic rat. *Cardiovasc Diabetol*. 2009;8:16.
32. Baldwin MD. Assessing cardiovascular risk factors and selecting agents to successfully treat patients with type 2 diabetes mellitus. *J Am Osteopath Assoc*. 2011;111:S2-S12.
33. Zhang CL, McKinsey TA, Chang S, Antos CL, Hill JA, Olson EN. Class II histone deacetylases act as signal-responsive repressors of cardiac hypertrophy. *Cell*. 2002;110:479-488.
34. Backs J, Song K, Bezprozvannaya S, Chang S, Olson EN. CaM kinase II selectively signals to histone deacetylase 4 during cardiomyocyte hypertrophy. *J Clin Invest*. 2006;116:1853-1864.
35. Antos CL, McKinsey TA, Dreitz M, et al. Dose-dependent blockade to cardiomyocyte hypertrophy by histone deacetylase inhibitors. *J Biol Chem*. 2003;278:28930-28937.
36. Kong Y, Tannous P, Lu G, et al. Suppression of class I and II histone deacetylases blunts pressure-overload cardiac hypertrophy. *Circulation*. 2006;113:2579-2588.
37. Duncan JG. Mitochondrial dysfunction in diabetic cardiomyopathy. *Biochim Biophys Acta*. 1813;2011:1351-1359.
38. Bruning JC, Michael MD, Winnay JN, et al. A muscle-specific insulin receptor knockout exhibits features of the metabolic syndrome of NIDDM without altering glucose tolerance. *Mol Cell*. 1998;2:559-569.

39. Pederson BA, Schroeder JM, Parker GE, Smith MW, DePaoli-Roach AA, Roach PJ. Glucose metabolism in mice lacking muscle glycogen synthase. *Diabetes*. 2005;54:3466-3473.
40. Kowalski GM, Bruce CR. The regulation of glucose metabolism: implications and considerations for the assessment of glucose homeostasis in rodents. *Am J Physiol Endocrinol Metab*. 2014;307:E859-E871.

SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

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