Genes controlling skeletal muscle glucose uptake and their regulation by endurance and resistance exercise

Sander A. J. Verbrugge1,2 | Julia A. Alhusen3 | Shimon Kempin2 | Nicolas J. Pillon4 | Jan Rozman5 | Henning Wackerhage2 | Maximilian Kleinert6,7

1Institute for Diabetes and Obesity, Helmholtz Diabetes Center (HDC), Helmholtz Zentrum München, Neuherberg, Germany
2Exercise Biology Group, Department for Sport and Health Sciences, Technical University of Munich, Munich, Germany
3Molecular Endocrinology, Institute for Diabetes and Cancer (IDC), Helmholtz Zentrum Munich, Helmholtz Diabetes Center (HMGU), Munich, Germany
4Department of Physiology and Pharmacology, Karolinska Institutet, Stockholm, Sweden
5Czech Centre for Phenogenomics, Institute of Molecular Genetics of the Czech Academy of Sciences, Vestec, Czech Republic
6Muscle Physiology and Metabolism Group, German Institute of Human Nutrition, Potsdam-Rehbrücke, Nuthetal, Germany
7Department of Nutrition, Exercise and Sports, Faculty of Science, Section of Molecular Physiology, University of Copenhagen, Copenhagen, Denmark

Correspondence
Sander Verbrugge, Institute for Diabetes and Obesity, Helmholtz Diabetes Center (HDC), Helmholtz Zentrum München, 85764 Neuherberg, Germany. Email: sander.verbrugge@helmholtz-muenchen.de
Henning Wackerhage, Department for Sport and Health Sciences, Exercise Biology, Technical University of Munich, Georg-Brauchle-Ring 60/62, 80992 München/Munich, Germany. Email: henning.wackerhage@tum.de
Maximilian Kleinert, Muscle Physiology and Metabolism Group, German Institute of Human Nutrition, Potsdam-Rehbrücke 14558 Nuthetal, Germany. Email: maximilian.kleinert@dife.de

Funding information
Deutsche Forschungsgemeinschaft, Grant/Award Number: KL 3285/2-1; CAS RVO, Grant/Award Number: 68378050; Czech Centre for

Abstract
Exercise improves the insulin sensitivity of glucose uptake in skeletal muscle. Due to that, exercise has become a cornerstone treatment for type 2 diabetes mellitus (T2DM). The mechanisms by which exercise improves skeletal muscle insulin sensitivity are, however, incompletely understood. We conducted a systematic review to identify all genes whose gain or loss of function alters skeletal muscle glucose uptake. We subsequently cross-referenced these genes with recently generated data sets on exercise-induced gene expression and signaling. Our search revealed 176 muscle glucose-uptake genes, meaning that their genetic manipulation altered glucose uptake in skeletal muscle. Notably, exercise regulates the expression or phosphorylation of more than 50% of the glucose-uptake genes or their protein products. This included many genes that previously have not been associated with exercise-induced insulin sensitivity. Interestingly, endurance and resistance exercise triggered some common but mostly unique changes in expression and phosphorylation of glucose-uptake genes or their protein products. Collectively, our work provides a resource of potentially new molecular effectors that play a role in the incompletely understood regulation of muscle insulin sensitivity by exercise.
1  INTRODUCTION

Skeletal muscle in humans contributes on average 38% and 31% to male and female body mass and stores 300–500 g of glucose in the form of glycogen.1,2 When insulin and glucose are co-infused during a hyperinsulinemic-euglycemic clamp, 70%–80% of the infused glucose is stored in skeletal muscle.3 This demonstrates the importance of skeletal muscle for blood glucose regulation. Conversely, insulin-resistant muscle takes up and stores less glucose per unit of insulin, requiring the pancreatic β cells to compensate by increasing insulin release. This results in hyperinsulinemia, a key characteristic of insulin resistance. Chronic skeletal muscle insulin resistance, therefore, strains the β-cells of the pancreas and is considered a key risk factor for developing type 2 diabetes mellitus (T2DM).4

Exercise increases glucose uptake into skeletal muscle of healthy and diabetic individuals.5 Generally, the more intense and the longer the exercise, the more glucose is taken up. Very intense exercise in humans can increase muscle glucose uptake 100-fold more than at rest.6 Notably, exercise-stimulated glucose uptake by muscle occurs independently of insulin signal transduction.7 This makes exercise an efficacious, non-pharmacological intervention to acutely decrease hyperglycemia in people with insulin resistance.8 In addition, exercise improves insulin-stimulated glucose uptake9–11 for 1–2 days after exercise.12,13 Such augmented muscle insulin sensitivity following a single bout of exercise is chiefly a local, contraction-induced effect. This is demonstrated by the robust increase in insulin sensitivity in perfused, electrically stimulated muscle compared to the unstimulated muscle of the contralateral leg in rats.14 Similarly, in humans following one-legged kicking exercise, the insulin sensitization is restricted to the exercised leg, with no improvements in the non-exercised leg.11,15 Following exercise-induced insulin sensitization, less insulin is required to lower postprandial glucose levels, reducing the burden on the pancreas to produce insulin. Engaging in regular exercise improves glycemic control and insulin action, and importantly, these benefits extent to patients with T2DM.16–19 In fact, these glucometabolic benefits of exercise are greater than those achieved by metformin, a widely prescribed antidiabetic drug in patients with T2DM.20 The implications of this are important, because it indicates that the molecular mechanisms that underpin exercise-induced skeletal muscle insulin sensitivity can bypass insulin resistance in sedentary patients. Despite these clear clinical benefits, the surprising fact is that the mechanisms for how exercise achieves this are incompletely understood.

Developments in systems biology have provided new tools to illuminate exercise signaling, with recent publications showing that exercise alters more than 3000 transcripts21 and 1000 protein phosphorylations22 in human skeletal muscle. While this demonstrates a hitherto unknown depth of exercise signaling, it also highlights the staggering task to understand the functional consequences of each of these molecular changes and to identify the ones that orchestrate improved skeletal muscle insulin sensitivity. To aid in the identification of such new regulators and pathways, we performed a systematic literature review to identify genes whose loss- or gain-of-function alters glucose uptake in mouse skeletal muscle. We then used existing transcriptome and phosphoproteome data sets on the effects of exercise, muscle contraction, and physical inactivity to investigate how these glucose-uptake genes and their protein products are regulated by exercise. This analysis revealed several previously unrecognized genes and proteins that are exercise-responsive and that alter glucose uptake. In addition, we observed that resistance and endurance exercise affect distinct genes and proteins regulating glucose uptake, suggesting that depending on the type of exercise there are several molecular pathways for improving insulin-controlled muscle glucose metabolism.

2  MATERIALS AND METHODS

2.1  Systematic literature search

We registered this systematic review on PROSPERO under access number CRD42019134929 (https://www.crd.york.ac.uk/PROSPERO/). To identify genes that regulate skeletal muscle glucose uptake, we carried out a systematic review according to the PRISMA guidelines23 and searched PubMed using the PICO framework24 leading to the following search strategy: ((("mice"[mesh] or "mouse model" or "mice, transgenic"[mesh] or "mice, knockout"[mesh] or "skeletal muscle" or "striated muscle" or "muscle")) and ("glucose uptake" or "glucose clearance" or "glucose metabolism" or "glucose tolerance" or...
“insulin sensitivity” or “glucose tolerance test”[mesh] or “glucose clamp technique”[mesh] or “glucose transport” or “glucose disposal” or “hyperinsulinemic clamp” or “euglycemic clamp” or “hyperinsulinemic-euglycemic clamp” or “oxidative glucose metabolism” or “non-oxidative glucose metabolism”) AND “muscle” AND “glucose uptake” NOT (review).

Note, we added ‘AND “muscle” AND “glucose uptake”’ to narrow down the number of studies to screen. Two researchers screened abstracts for eligibility that were indexed in PubMed until March 23, 2020. In addition, one researcher searched the Mouse Genome Informatics (MGI) database (http://www.informatics.jax.org) and included references associated with “increased muscle cells glucose uptake” (ID: MP0030021) or “decreased muscle cell glucose uptake” (ID: MP0030022). We included articles from peer-reviewed journals, written in English, that studied skeletal muscle glucose uptake in gene-manipulated mouse models in vivo, as measured by 2-deoxyglucose (2DG) uptake. We excluded studies where one or more of the following applied:

- Retracted article.
- Article in other languages than English.
- More than one intervention (e.g., double gene manipulation).
- Rat or in vitro study.
- No use of wildtype or other control group.
- Mice on ob/ob or db/db background.
- No effect on skeletal muscle glucose uptake.
- 14C enrichment or FDG-PET as measure of glucose uptake.
- Not first study reporting an effect of particular gene manipulation on glucose uptake.

Three researchers read full-text articles and extracted the following information:

- Author,
- Official mouse gene name,
- Mouse aliases from Uniprot,
- Human aliases from Uniprot,
- Protein name from Uniprot,
- Details of gene manipulation,
- Global or tissue-specific gene manipulation,
- Gain- or loss-of-function,
- Sex of experimental animals,
- Number of tested animals,
- Percentage difference in 2DG uptake between transgenic and control mice in soleus, extensor digitorum longus, tibialis anterior, gastrocnemius, quadriceps, or “other” muscle,
- In vivo or ex vivo,
- Increase or decrease in skeletal muscle mass.

We compiled this information in Table S1. The difference in glucose uptake between transgenic and control mice was often not quantified in the articles we assessed, but only depicted in bar graphs. In that case, we manually estimated the relative difference between transgenic and control mouse from the bar graph. Note, we adopted the official gene name from Uniprot, which could vary from an alias used in the original publication.

2.2 | Bioinformatical analysis

2.2.1 | MetaMEx

We used MetaMEx to study whether glucose uptake genes change their expression in human skeletal muscle in response to acute aerobic and resistance exercise, aerobic and resistance training, or inactivity.21 The MetaMEx database was parsed for exercise and inactivity studies in healthy male and female volunteers. Studies in athletes, individuals with diagnosed diseases, or individuals with obesity were excluded. In acute exercise studies, only the data from biopsies collected 0–6 h after exercise was selected. Meta-analysis was calculated in R 4.0.1 using a random effect model with the metafor package.

2.2.2 | Phosphoproteomics following exercise and muscle contraction

To compare whether proteins encoded by glucose uptake genes are phosphorylated or dephosphorylated in skeletal muscle after exercise in humans, we overlapped the supplementary data from Nelson et al.25 with our identified glucose uptake genes and checked for altered phosphorylation of glucose uptake proteins in response to treadmill exercise and electrically stimulated muscle contractions in rodents.

Figures were created with GraphPad Prism and with BioRender.com.

3 | RESULTS

We screened 5597 abstracts from Pubmed and the Mouse Genomics Informatics database. After removing duplicates and applying exclusion criteria, we read 372 articles full-text of which 171 matched our eligibility criteria (see Methods). A PRISMA flowchart on search and selection of eligible articles is illustrated in Figure 1.
From 171 articles, we identified 176 genes whose manipulation changed glucose uptake in skeletal muscle. Gain-of-function of 32 genes increased glucose uptake, while 24 decreased glucose uptake. Loss-of-function of 67 genes increased glucose uptake, whereas for 67 this decreased glucose uptake. For simplicity, we refer from now on to the 176 identified genes as well as to the proteins encoded by them as “glucose-uptake genes.” In Figure 2, we plotted the relative changes in glucose uptake between wildtype and transgenic mice for basal, insulin- and contraction-stimulated glucose uptake. Manipulation of genes alters basal glucose uptake from a 330% increase (Slc2a1, which encodes GLUT1) to a 70% decrease (Sik2) (Figure 2a,b). Changes in insulin-stimulated glucose uptake after gene manipulation ranged from +313% (Itga2) to −70% (Hdac3) (Figure 2c,d). In addition, we identified several genes that affect skeletal muscle glucose uptake during muscle contractions or exercise. Here, the mutation-induced effect sizes range from a 75% increase (Glp1r) of glucose uptake to a 65% decrease (Chga) (Figure 2e,f).

To identify potential novel regulators of exercise-induced insulin sensitivity, we compared the 176 glucose-uptake genes with recent studies on global gene and signaling changes in response to exercise or inactivity in human and rodent skeletal muscle. Figure 3 shows glucose-uptake genes with changed expression in human muscle biopsies collected 0–6 h following acute aerobic exercise (cycling at various intensities and different durations) and acute resistance exercise (mostly knee extension exercise). For details of exercise protocols, see https://www.metamex.eu. Overall, acute exercise altered 63 glucose-uptake genes. Notably, of these 63 genes, both exercise types—resistance versus endurance—regulated only 19 genes commonly. This indicates distinct molecular pathways for how aerobic vs. resistance-type exercise alters insulin sensitivity (Figure 3a,c,d). Exercise training (i.e., repeated bouts of exercise over several weeks) resulted in less pronounced changes, with 15 genes increased and 15 genes decreased (Figure S1a,b). Adaptions to the exercise stimulus that occur with training likely explain this blunted response.

**FIGURE 1** PRISMA flowchart on search and selection of eligible articles
FIGURE 2 Changes in glucose uptake in mice with gene gain- or loss-of-function. Changes in (A, B) basal, (C, D) insulin-stimulated, and (E, F) contraction/exercise-stimulated glucose uptake in transgenic mice compared to controls. LOF, loss-of-function (red), GOF, gain-of-function (green). Some studies tested both gene LOF and GOF (Tpm3, Nrip1, Dgat1, Appl2). Note that the manipulation of the same gene can lead to both an increased and decreased glucose uptake depending on the testing conditions (basal vs. insulin-stimulated vs. contraction-stimulated) (Cnr2, Rac1, Tbc1d1, Igfbp1, Dgat2, and Rxrg)
Increased sedentary time and physical inactivity are associated with a greater risk for developing T2DM and the metabolic syndrome, while physical activity reduces this risk for developing T2DM. Bed rest or limb immobilization, two experimental models for inactivity, altered the expression of 34 glucose-uptake genes in human skeletal muscle. Of those, 19 increased, while 16 glucose-uptake genes were decreased (Figure 3b). Notably, exercise reciprocally regulates ~40% of these genes, highlighting that exercise can quickly reverse the molecular fingerprint of physical inactivity in skeletal muscle (Figure S1c).

In addition to gene expression changes, exercise rapidly and comprehensively changes protein phosphorylation which is a key mode of signal transduction and metabolic regulation. For instance, more than 560 proteins are differentially phosphorylated in human muscle after a vigorous bout of cycling exercise. We found that in humans, exercise alters 59 phosphorylation sites on 29 unique proteins mapping to the glucose-uptake genes. Most of these proteins exhibited increased phosphorylation sites following exercise, while nine proteins were dephosphorylated (PPP1R3C, PRKAG3, AKT2, ILK1, FOXO1, FOXO3, RICTOR, CRTC3, and PTPN11). Five proteins, ANK2, IRS1, CSRP3, PPP1R3A, and TBC1D4, contain both increased and decreased phosphorylation sites (Figure 4a). The latter, TBC1D4, is an established regulator of glucose uptake in skeletal muscle that is modulated by both exercise and insulin stimulation. In rodents, treadmill running altered the phosphorylation of 50 sites on 24 proteins encoded by glucose-uptake genes (Figure 4b). Electric-stimulated in situ muscle contractions in rats altered the phosphorylation of 63 sites on 22 proteins encoded by glucose-uptake genes (Figure 4c). Similar to the data in humans, the phosphorylation of most sites increased following exercise/contractions in rodent muscle.

Taken together, exercise and muscle contractions regulate the phosphorylation on 40 sites of proteins that are encoded by glucose-uptake genes, 21 of which are also transcriptionally regulated by exercise or physical inactivity (Figure 3). Overall, transcriptomic and phosphoproteomic analyses reveal that acute exercise regulates ~50% of the glucose-uptake genes or their protein products in skeletal muscle.

To identify those glucose-uptake genes that have not been previously identified as exercise targets, we parsed them through a systematic PubMed search ([(gene name OR aliases OR “protein names” AND “exercise”)]. As an overview, we integrated all the exercise-regulated glucose-uptake genes (or their protein products) by exercise type (resistance vs. endurance), which emphasizes the distinct regulation by exercise type (Figure 5).

As expected, some hits are well known to be exercise-responsive. These include GLUT4, AMPK (PRKAB1, PRKAB2, PRKAA1, PRKAA2), TBC1D1, and TBC1D4. Additionally, our analysis uncovered glucose-uptake genes that have not previously been linked to exercise (indicated with * in Figure 5). These include DOC2B, STX4, and PEA15, which all have been implicated in the regulation of GLUT4 translocation to the plasma membrane. Similarly, TIMP3 and CEACAM1 are regulators of angiogenesis and thus could play an important role in delivering glucose to muscle, which is one of the key determinants regulating muscle glucose uptake in vivo. STEAP4, HFE, ABC9, and RRAD regulate iron metabolism. Iron metabolism is connected to insulin action, as high tissue iron levels are correlated with insulin resistance; and notably, exercise is thought to decrease iron stores in the muscle.

We also identified the transcription factors PKNOX1, ID2, and HMGA1, and the protein kinases SIK1, SPHK1, and DMPK as new exercise-responsive glucose-uptake genes. HMGA1 is particularly interesting because this transcription factor and epigenetic modifier regulate genes involved in glucose metabolism. For instance, HMGA1 controls the expression of the insulin receptor and lack of HMGA1 is associated with insulin resistance and diabetes in humans and mice. Its regulation by exercise warrants clarification.

4 | DISCUSSION

We combined a systematic literature review with analyses of data sets on exercise-induced gene expression and signaling and identified 176 genes that alter muscle glucose uptake. Roughly 50% of these genes are also regulated by exercise through transcription or phosphorylation in muscle. These exercise-responsive glucose-uptake genes comprise several known regulators of exercise-mediated glucose uptake such as calmodulin, CAMKKII, LKB1, AMPK, PKC, RAC1, and GLUT4. We also found that exercise regulates members of the insulin-signaling cascade including TBC1D1, TBC1D4, IRS1, IRS2, FOXO1, and AKT2, highlighting a comprehensive interplay between exercise and insulin signaling that might contribute to how exercise increases insulin sensitivity. We also uncovered several new links between exercise-responsive and muscle glucose-uptake genes as highlighted in Figure 5, which includes candidates for the elusive regulators of improved insulin sensitivity following exercise.

We found 17 glucose-uptake genes that preferentially respond to endurance exercise and 27 glucose uptake genes that specifically respond to resistance exercise.
This suggests that endurance and resistance exercise could improve glycemic control and insulin sensitivity in T2DM by similar but also unique signaling mechanisms. An exercise type-specific regulation of insulin sensitivity would provide several pathways that could be targeted to bypass insulin resistance in sedentary people. It also raises the question whether DNA variations of some of these genes explain why some people respond better to one type of exercise for improving glycemic control and treating T2DM.

Skeletal muscle is a heterogeneous tissue composed of type 1, 2a, 2x (2b in rodents) fiber types as well as other...
cell types. Broadly speaking, slow-twitch type 1 fibers are less powerful but more fatigue resistant. In contrast, fast-twitch type 2a and 2b fibers are more powerful but fatigue quicker. It has been suggested that the regulation of glucose uptake differs among fiber types. Specifically, Albers et al. suggested that human type 1 fibers have the molecular machinery for a higher glucose-handling capacity when compared with type II fibers. This is important for the interpretation because some of the rodent studies we assessed measured glucose uptake in both soleus (type 1 fiber dominant) and EDL or TA (type 2 fiber dominant) muscle and found that basal glucose uptake, insulin-stimulated glucose uptake, and exercise-induced glucose uptake were differently affected by gene manipulation. Unsurprisingly, soleus and EDL muscles express many genes differently and single fiber proteomics uncovered fiber-type specific features, including differences in the abundance of proteins related to mitochondrial and substrate utilization.

Endurance exercise like cycling and running involves prolonged periods of low loads, recruitment of smaller motor units comprising mostly type 1 fibers, and a lower firing rate. Resistance exercise, on the other hand, comprises higher loads, recruitment of larger motor units comprising both type 1 and 2, and higher firing rates. This might explain differences in gene expression as a result of endurance and resistance exercise. Furthermore, exercise training triggers muscle fiber-specific adaptations: Following twelve weeks of aerobic training, the abundance of 205 proteins was altered in type 1 fibers, and 140 proteins were changed in type 2 fibers. However, of those, only 32 proteins were changed in both fiber types in response to aerobic training, suggesting that the majority of exercise-stimulated protein adaptations are fiber type-specific.

One intriguing question is whether resistance training-induced hypertrophy includes a cancer-like metabolic reprogramming similar to the Warburg effect. During such metabolic reprogramming glucose and energy metabolites are more channeled into anabolic reactions such as nucleotide, amino acid, or phospholipid synthesis. We have previously proposed that an elevated glucose uptake during acute resistance exercise provides carbon for synthesizing amino acids, nucleotides, and lipids to support anabolism in hypertrophying muscles. Recently, glycolysis has been shown to limit basal protein synthesis suggesting that glucose metabolism plays a role in the maintenance of muscle mass. The manipulation of several glucose-uptake genes is associated with regulating muscle hypertrophy and glucose uptake in

FIGURE 4 (See caption on next page)
concert. For example, MSTN loss-of-function is known to increase muscle mass, while simultaneously increasing glucose uptake. Other examples are Foxc2, Fst, Ccn5, Cpt1b, Hdac3, Insr, Steap4, Igfbp3, Tnfsf11, Inpp5k, and Nos2, whose gene manipulation affect both muscle weight and glucose uptake in mice.

Another question is, how to best assess insulin sensitivity in muscle after exercise in mice. There are several options. The hind-limb perfusion model allows the experimenter to assess muscle glucose uptake in response to different concentrations of insulin and glucose in vivo. This model was
used when Richter et al. demonstrated for the first time that exercise improves muscle insulin sensitivity in rats.\textsuperscript{10} However, the smaller anatomy of the mouse makes hindlimb perfusion more challenging. The combination of a hyperinsulinemic-euglycemic clamp and radioactive glucose tracers allows for determination of whole-body and skeletal muscle insulin sensitivity in rodents.\textsuperscript{83} Steady infusion of glucose and insulin require surgical implantation of catheters. These surgeries can be conducted several days before experiment, but then the catheters can interfere during the exercise bout. Alternatively, catheters can be put in right after exercise, but prolonged anesthesia can induce insulin resistance,\textsuperscript{84} confounding the effects of exercise. Two additional methods are worth considering. One, retro-orbital injection of a bolus comprising a physiological dose of insulin, glucose, and radioactive glucose tracers. With this method, it was shown that skeletal muscle insulin sensitivity was increased following prior treatment with the AMPK agonist AICAR.\textsuperscript{85} Interestingly, to our knowledge, this method has not been used to assess skeletal muscle insulin sensitivity after exercise. One caveat is that mice also have to be anesthetized for this procedure, but the duration of anesthesia is relatively short. Two, the ex vivo isolated muscle incubation technique can be performed following exercise. For example, Serup et al. found that ex vivo insulin-stimulated glucose uptake was augmented in EDL and soleus muscles from HSL KO mice 90 min after treadmill running.\textsuperscript{86} One limitation is that the insulin-sensitizing effect on glucose uptake is moderate with this technique, likely making it difficult to detect decreases in insulin sensitivity following exercise.\textsuperscript{87}

We have compiled all genes whose manipulation changes skeletal muscle glucose uptake in mice. This approach has some limitations. We did not distinguish between the effects of global and tissue-specific gene manipulation. Tissue-specific gene gain- or loss-of-function in skeletal muscle likely affects skeletal muscle glucose uptake directly. For mouse models that have a global gene manipulation or specifically in non-muscle tissue, the regulation of glucose uptake in skeletal muscle could be through indirect mechanisms. Nevertheless, mouse models with gene manipulation in non-muscle tissues still identify targets that are expressed in skeletal muscle and that are altered in response to acute exercise (e.g., LRP1, BCL6, TP53, CAT, TIMP3, HMGA1, CEACAM1) in human skeletal muscle biopsies.\textsuperscript{21}

In summary, both endurance and resistance exercise mitigate skeletal muscle insulin resistance.\textsuperscript{88} Combining aerobic and resistance exercise might offer superior effects on glucose homeostasis compared to aerobic or resistance exercise alone,\textsuperscript{89–91} as each type of exercise regulates some glucose-uptake genes that are not regulated by the other. Our analysis provides a molecular framework for how these two types of exercise elicit improvements in glucose homeostasis as they seemingly engage distinct pathways. Considering the natural variation in muscle fiber type distribution in human skeletal muscle,\textsuperscript{92} our work provides a resource to develop novel targeted and personalized treatment strategies for improving glucose uptake and bypassing insulin resistance in patients with T2DM.

ACKNOWLEDGMENTS

JR and Czech Centre for Phenogenomics are supported by the CAS RVO 68378050 and by the project LM2018126 Czech Centre for Phenogenomics provided by MEYS. MK is supported by the Deutsche Forschungsgemeinschaft (KL 3285/2-1) and the Novo Nordisk Foundation (NNF19OC0055192). Open Access funding enabled and organized by Projekt DEAL.

CONFLICT OF INTERESTS

The authors declare that there are no conflict of interests.

AUTHOR CONTRIBUTIONS


DATA AVAILABILITY STATEMENT

The data that supports the findings of this study are available in the supplementary material of this article.

ORCID

Sander A. J. Verbrugge \(\text{https://orcid.org/0000-0001-7531-7879}\)
Nicolas J. Pillon \(\text{http://orcid.org/0000-0003-1107-9490}\)
Maximilian Kleinert \(\text{http://orcid.org/0000-0002-8069-9055}\)

REFERENCES


73. Grüning JR, Hoffmann JM, Hedjazifar S, et al. Over-expressing the novel autocrine/endocrine adipokine WISP2...


**SUPPORTING INFORMATION**

Additional supporting information may be found in the online version of the article at the publisher’s website.