The exercise-inducible bile acid receptor Tgr5 improves skeletal muscle function in mice

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Running title: Functions and expression mechanism of TGR5 in muscle

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Keywords: skeletal muscle, bile acid, muscle hypertrophy, unfolded protein response, UPR, ER stress, transcription regulation, muscle cell differentiation, TGR5, GPBAR1

Abstract

TGR5 (also known as G protein–coupled bile acid receptor 1, GPBAR1) is a G protein–coupled bile acid receptor that is expressed in many diverse tissues. TGR5 is involved in various metabolic processes, including glucose metabolism and energy expenditure; however, TGR5’s function in skeletal muscle is not fully understood. Using both gain- and loss-of-function mouse models, we demonstrate here that Tgr5 activation promotes muscle cell differentiation and muscle hypertrophy. Both young and old transgenic mice with muscle-specific Tgr5 expression exhibited increased muscle strength. Moreover, we found that Tgr5 expression is increased by the unfolded protein response (UPR), which is an adaptive response required for maintenance of endoplasmic reticulum (ER) homeostasis. Both ER stress response element (ERSE) and unfolded protein response element (UPRE)-like sites are present in the 5′ upstream region of the Tgr5 gene promoter and are essential for Tgr5 expression by activating transcription factor 6α (Atf6α), a well-known UPR-activated transcriptional regulator. We observed that in the skeletal muscle of mice, exercise-induced UPR increases Tgr5 expression, an effect that was abrogated in Atf6α KO mice, indicating that Atf6α is essential for this response. These findings indicate that the bile acid receptor Tgr5 contributes to improved muscle function and provide an additional explanation for the beneficial effects of exercise on skeletal muscle activity.

Bile acids are essential for solubilizing lipids and fat-soluble vitamins in order to promote their absorption in the small intestine. In addition to these classical functions, bile acids are known to function as metabolic regulators (1). In the postprandial state, bile acids are reabsorbed in the ileum and the bile acid concentration temporarily reaches a high level in blood, then bile acids are transported back to the liver and recycled. This cycle called enterohepatic circulation is achieved by bile acid transporters which are exclusively expressed in the ileum and liver. TGR5 (also known as GPBAR1) is the only G protein-coupled receptor that has bile acids as its ligand. TGR5 is expressed in multiple tissues, such as brown adipose tissue (BAT), small intestine and skeletal muscles (2,3). Ligand-bound TGR5 induces intracellular cAMP production via the Gαs subunit, resulting in the
activation of the protein kinase A (PKA)–cAMP response element-binding protein (CREB) pathway (2-4), which regulates numerous biological processes (5). Previous reports have shown that TGR5 activation in enteroendocrine L cells and colon stimulates glucagon-like peptide-1 (GLP-1) release and improves glucose homeostasis (6-9). Furthermore, TGR5 activation triggers energy expenditure via type 2 deiodinase (Dio2) expression in human and mouse BAT, as well as in primary myotubes of human skeletal muscle. However, this has not been observed in mouse skeletal muscle, because of extremely low expression of Dio2 (4,10).

Recent studies indicated that bile acid synthesis is increased by cold exposure in mice, and elevated bile acid circulating causes adaptive thermogenesis, possibly by BAT activation and beiging of subcutaneous white adipose tissue (WAT) (11,12). Therefore, TGR5 activation shows promise for the treatment of diabetes, obesity, and associated metabolic disorders; however, the functions of TGR5 in skeletal muscle are not well understood.

The endoplasmic reticulum (ER) is an evolutionarily conserved subcellular organelle that regulates protein synthesis, folding, and assembly. ER stress, which is defined as the cell state in which ER accumulates unfolded or misfolded proteins, occurs when ER function is disrupted, and causes metabolic disorders such as obesity and type 2 diabetes (13,14). Cells that sense ER stress activate the unfolded protein response (UPR), which involves three major ER stress sensors: protein kinase R-like endoplasmic reticulum kinase (PERK), inositol-requiring enzyme 1 (IRE1), and activating transcription factor 6α (ATF6α) (15). To increase ER protein folding capacity and attenuate ER stress, activated UPR sensors produce a variety of transcription factors, such as ATF4, the spliced form of XBP-1 (XBP-1s), and the N-terminal of cleaved ATF6α (ATF6α(N)). Previous studies have shown that exercise stimulates the UPR in human and mice skeletal muscles, and, importantly, UPR activation helps skeletal muscles adapted to exercise (16,17).

Furthermore, another recent report demonstrated that inhibition of ER stress leads to muscle wasting in Lewis lung carcinoma and ApcMin/+ models of cancer cachexia, as well as naïve mice, indicating that ER stress and UPR pathways contribute to maintain skeletal muscle mass and strength (18). Taken together, ER stress is not always harmful but, rather, appears to assist in multiple physiological processes in skeletal muscle.

In the present study, we investigate the role of TGR5 in the control of skeletal muscle function. Interestingly, Tgr5 KO mice exhibited lower muscle mass and strength, and decreased the expression of several muscle hypertrophy and differentiation-related genes in skeletal muscle. As expected from these findings, TGR5-expressing C2C12 myoblasts and muscle-specific TGR5 transgenic (Tg) mice clearly show that TGR5 activation enhances muscle cell differentiation and muscle hypertrophy. Furthermore, we identify Tgr as a novel UPR target gene and reveal that exercise induces muscle Tgr5 expression in an Atf6α-dependent manner. These findings show that TGR5 plays an important role in regulating muscle function and provide a new explanation for the beneficial effects of exercise.

Results

Tgr5 KO mice exhibit lower muscle mass and strength

Since TGR5 is known to activate CREB via cAMP production (2,3), we measured the mRNA expression of CREB target genes in the skeletal muscle of Tgr5 KO mice to evaluate the function of muscle Tgr5 in vivo (5,19) (Fig. 1A). As expected, gene expressions of several CREB targets were decreased in the soleus of Tgr5 KO mice compared with wild-type (WT) littermates (Fig. 1A). Decreased genes included salt-inducible kinase 1 (Sik1), which plays an important role in muscle cell survival and differentiation (20,21), and the hypertrophy-related genes Nrr4α1 and Pgc-1α4 (22,23). We also observed increased mRNA expression of the muscle atrophy-related E3-ubiquitin ligases Musa1 and Smart in Tgr5 KO mice (24,25) (Fig. 1A). These findings indicate that TGR5 works as a muscle mass regulator.
There were no differences in body weights between WT and Tgr5 KO mice under the standard chow diet, which is compatible with a previous report (26) (Fig. 1B). However, Tgr5 KO mice exhibited lower soleus and quadriceps mass compared to WT littermates (Fig. 1C). The same results applied to the ratio of muscle weights to body weight (Fig. 1D). Consistent with these findings, Tgr5 KO mice showed significantly lower strength than WT littermates (Fig. 1E). These data demonstrate that TGR5 is involved in regulating muscle mass and strength in vivo. Next, we measured food intake, energy expenditure, the respiratory exchange ratio (RER), and locomotor activity in Tgr5 KO and WT mice. Energy expenditure and RER were calculated from O2 consumption (VO2) and CO2 production (VCO2) by using a mass spectrometer. We found no differences in these measurements, showing that muscular atrophy observed in Tgr5 KO mice was not caused by changes in energy metabolism or physical activity (Fig. 1, F–J).

**TGR5 enhances muscle cell differentiation in C2C12 myoblasts**

SIK1 phosphorylates class II histone deacetylases, resulting in the activation of myocyte enhancer factor 2 (MEF2), which is considered to act as a linchpin for muscle development and differentiation (20,27). Therefore, based on our current findings that Tgr5 KO mice had decreased Sik1 expression and muscle mass, we aimed to determine the effect of TGR5 activation on muscle differentiation. Because of low Tgr5 expression in C2C12 myoblasts, we use hTGR5 over-expressing C2C12 myoblasts and confirmed that TGR5 activation with tauroliothocholic acid (TLCA), one of the most potent endogenous ligands for TGR5, induces CRE reporter activation (Fig. S1A and Fig. 2A). In addition, TLCA treatment in C2C12 myoblasts over-expressing TGR5 with adenovirus increased the mRNA levels of Nr4a2, a known CREB responsive gene, and Sik1 (2.7-fold and 3.5-fold, respectively), whereas TLCA treatment in LacZ-expressing C2C12 myoblasts only slightly increased these mRNA levels (1.2-fold each) (Fig. 2B). Similar results were also observed when using cholic acid (CA) or deoxycholic acid (DCA) instead of TLCA (Fig. S1, B and C). To evaluate the effect of TGR5 on muscle differentiation, LacZ or TGR5 over-expressing C2C12 myoblasts were differentiated with or without TLCA, and the mRNA levels of Sik1 and several differentiation marker genes, including MyoD, Myogenin, Muscle creatine kinase (MCK), Mef2a, and MyHC, were measured. TGR5/LCXA C2C12 myoblasts showed the highest mRNA expression levels of Sik1 and differentiation marker genes, particularly on day 2 (48 h after TLCA treatment) (Fig. 2C). The measurement of the differentiation index (i.e., the percentage of nuclei in MyHC-positive cells above total nuclei) also revealed that TLCA significantly promoted the differentiation of TGR5-expressing C2C12 myoblasts (1.5-fold). LacZ-expressing C2C12 myoblasts showed a similar tendency (1.3-fold), although it was not statistically significant (Fig. 2D). Endogenous Sik1 silencing using two types of siRNA attenuated the mRNA expression of several muscle differentiation marker genes in TGR5/LCXA C2C12 myotubes, indicating the importance of Sik1 for TGR5-mediated muscle differentiation (Fig. 2E, the details of statistical analysis were shown in Table S1). These results demonstrated that TGR5 activation leads to muscle cell differentiation due, in part, to the upregulation of Sik1 expression.

**TGR5 induces muscle hypertrophy in mice**

To evaluate the function of TGR5 in differentiated mouse skeletal muscle, we generated muscle-specific Flag-hTGR5 Tg mice under the control of the MCK promoter and enhancer. Among four transgenic lines obtained (lines A–D), line A and B showed higher Flag-hTGR5 expression levels than lines C and D (Fig. S2A). Thus, unless otherwise noted, line A was used in the following experiments. Flag-hTGR5 expression was observed in skeletal muscles but not in the heart, liver, kidney, epididymal WAT, or BAT (Fig. S2B). Flag-hTGR5 was strongly expressed in white muscle tissue such
as that of the gastrocnemius (gastro) and quadriceps (quad), whereas it was weakly expressed, as expected, in the soleus, a typical red muscle, using the MCK promoter (Fig. S2C).

We observed no differences in the daily food intake between Tg mice and WT littermates fed a normal diet at 8 weeks old (Fig. 3A). However, Tg mice were slightly but significantly heavier (Fig. 3B). Consistent with our finding that Tgr5 KO mice have muscle atrophy, Tg mice exhibited a 9.5% and 14.6% increase in gastrocnemius and quadriceps mass, respectively, where sufficient levels of Flag-hTGR5 protein were found (Fig. 3, C and D, and Fig. S2C). The same results applied to the ratio of gastrocnemius and quadriceps weights to body weight (7.5% and 12.5% respectively) (Fig. S2D). In contrast, tissue weights did not increase in the soleus, liver, or WAT (Fig. 3C). Similar changes in body and tissue weights were also seen in female Tg mice (Fig. S2, E and F). In addition, the quantitative analysis of gastrocnemius fiber size revealed an increased average cross-sectional area in Tg mice (Fig. 3, E and F); particularly, TGR5 appeared to drive an increase in the frequency of very large fibers (>2400 μm²), which was uncommon in WT littermates. As expected, this muscle mass increment was accompanied by an increase in grip strength in Tg mice at 2, 6, 9, 12, 15, 18 and 21 months old (Fig. 3G).

However, since there was no difference in the degree of muscle weakness due to aging and muscle atrophy caused by denervation, TGR5 seems to increase muscle mass by inducing muscle hypertrophy rather than suppressing muscle atrophy (Fig. 3G and Fig. S2G). Intramuscular TLCA injection to the quadriceps of Tg mice immediately promoted the mRNA expression of Siki and the hypertrophy-related genes Nr4a1 and Pgc-1a4, which were decreased in the skeletal muscle of Tgr5 KO mice (Fig. 3H and Fig. 1A). These responses were not strong in WT littermates; however, we observed a mild increment of these genes 3 h and 8 h after TLCA injection. Previous reports have shown that NR4A1- and PGC-1α4-induced muscle hypertrophy is mediated by the growth-promoting gene IGF1, and growth-limiting genes, such as Myostatin, Atrogin1, and MuRF1 (22,23). Consistent with these reports, TLCA injected Tg mice showed significantly higher IGF1 and lower Atrogin1 mRNA expressions compared with the vehicle group 8 h after injection, although Myostatin and MuRF-1 were not affected. Moreover, Musa1, which is required for protein breakdown in atrophying muscles, was negatively regulated by TGR5 activation in contrast to Tgr5 KO mice (24) (Fig. 3H and Fig. 1A).

Phosphorylation of Akt was also accelerated as IGF1 expression increased (Fig. 3J). These results suggest molecular evidence that activation of TGR5 in matured skeletal muscle induces muscle hypertrophy and increases muscle strength.

To evaluate the possibility that TGR5 also promotes muscle hypertrophy in humans, TGR5 over-expressing human skeletal muscle myotubes were treated with TLCA, and mRNA expression levels were measured. TGR5 activation increased SIK1, NR4A1, NR4A3, PGC-1α4, and IGF1 mRNA expression, as well as these in TLCA treated Tg mice, and decreased Myostatin, MuRF1, and MUSA1 mRNA expression significantly in human skeletal muscle myotubes (Fig. 4). We next performed an analysis of gene expression in human skeletal muscle using RNA-sequence data from the Genotype-Tissue Expression project (28). Interestingly, TGR5 mRNA expression showed moderate positive correlations with that of IGF1 (Pearson’s r = 0.354), although very weak or no correlations were observed with that of muscle atrophy-related E3 ligases and Myostatin (|r| < 0.2) (Fig. S3). These data suggest that TGR5 has the potential to regulate muscle mass in humans similar to mice.

**Tgr5 expression was upregulated by the UPR in muscle cells**

As observed above, alterations in TGR5 expression level had effects on muscle mass and muscle cell differentiation. However, little is known regarding the molecular mechanisms that regulate endogenous TGR5 expression until now. Interestingly, we found that thapsigargin (an ER-specific calcium ATPase
inhibitor) and tunicamycin (an N-glycosylation inhibitor), both well-known UPR inducers, increased Tgr5 mRNA levels in C2C12 myotubes (2.1- and 2.5-fold, respectively) similarly to several UPR marker genes, including Atf4, Atf6α, Bip, total Xbp-1 (Xbp-1), and Xbp-1s (Fig. 5A). The increase in Tgr5 and Bip mRNA expression by UPR inducers was accompanied by their increased protein levels (Fig. 5B). A similar response was observed in mouse skeletal muscle when thapsigargin was intramuscularly injected (1.6-fold for Tgr5) (Fig. 5C). Consistent with these results, a reporter gene assay using the mouse Tgr5 (mTgr5) promoter region also showed that the promoter activity was increased by tunicamycin treatment (2.5-fold) (Fig. 5D). These results strongly suggest that Tgr5 is a novel UPR target gene.

**Atf6α activates Tgr5 transcription and is necessary for exercise-induced Tgr5 expression**

UPR, which involves the ATF6α, IRE1, and PERK pathways, is known to activate various transcription factors such as ATF2, ATF3, ATF4, ATF6α, CHOP, and XBP-1. To identify the direct factors that regulate TGR5 transcription, we performed reporter assays on the mTgr5 promoter, and found that ATF6α(N) and XBP-1s were potent factors that promote mTgr5 promoter activity (Fig. 6A). A previously published report showed that both ATF6αa and XBP-1 bind to the ER stress response element (ERSE) in an NF-Y-dependent manner, while XBP-1 also binds to an unfolded protein response element (UPRE) without NF-Y (29). Consistent with this report, there is a combined ERSE-like and UPRE-like element in the 5′ upstream region of the mTgr5 promoter (Fig. S4). Interestingly, the assay on the mTgr5 promoter with mutations in these elements indicated that the 5′-GCAGT-3′ sequence, a potential NF-Y-binding site, and the 5′-CCACG-3′ sequence, a potential ATF-6a- and XBP-1-binding site, were necessary for ATF6α(N)-induced mTgr5 promoter activation (Fig. S4 and Fig.6B). In contrast, XBP-1s responsive promoter activation was strongly but not completely eliminated by the mutation in the 5′-GCAGT-3′ sequence, while

the unaltered 5′-CCACG-3′ sequence was essential for this response (Fig. S5). These findings suggest that ATF6α(N) binds to the ERSE-like element, while XBP-1s recognizes both the ERSE-like and UPRE-like elements in the mTgr5 5′ upstream region. To test whether ATF6αa(N) increases endogenous Tgr5 expression, C2C12 myotubes were transduced with adenovirus-expressing ATF6αa(N). We observed a significant increase in Tgr5 mRNA (16.3-fold) in ATF6αa(N)-expressing C2C12 myotubes, which was higher than that of Bip (6.9-fold), a major ATF6α target gene (Fig. 6C). Similarly, these protein levels were also increased by ATF6αa(N) over-expression (Fig. 6D). A CRE-Luc assay revealed that ATF6αa(N)-expressing C2C12 myoblasts reacted to TLCA more strongly than LacZ-expressing C2C12 myoblasts (Fig. 6E).

Exercise has been reported to activate the UPR in mouse and human skeletal muscles (16,17). Particularly, exercise-induced PGC-1α promotes UPR gene expression, in part by coactivating ATF6α (17). Indeed, we found that ATF6αa and PGC-1α coordinately activated the mTgr5 promoter (Fig. 6F). Therefore, we subjected WT and Atf6αa KO mice to treadmill running and analyzed gene expression in their quadriceps. The mRNA levels of Tgr5 and UPR marker genes increased significantly in WT mice after exercise (1.4-fold for Tgr5) (Fig. 6G). In contrast, the induction of Tgr5 and some UPR marker genes, such as Bip and Chop were completely repressed in Atf6α KO mice. These observations indicate that Tgr5 is a novel UPR target gene, and Atf6α is necessary for exercise-induced increases in muscle Tgr5 expression.

**Discussion**

Ligand-bound GPCRs activate specific signaling pathways depending on the type of coupled G-protein α-subunit. Since TGR5 is coupled with Gnas, its activation enhances the cAMP-PKA-CREB pathway via adenylyl cyclase in various types of cells (2,4,30). Previously, several Gnas-coupled GPCRs, such as β-adrenergic receptor (β-AR), Frizzled7 and CRFR2, were shown to induce muscle
hypertrophy and attenuate muscle atrophy (31). For example, the prolonged administration of β-AR agonists increases muscle mass and attenuates sarcopenia (32,33). β-AR mediated muscle hypertrophy requires CREB and its coactivators, which regulate muscle mass regulatory genes, such as PGC-1α, SIK1, and NR4A1 (34). Indeed, silencing Pgc-1α has been shown to blunt β-AR agonist-induced muscle hypertrophy in mouse primary myotubes (23). Consistent with these findings, the genetic deletion of Gα in skeletal muscle decreases total Pgc-1α expression and induces muscle atrophy (35). Another study showed that muscle-specific expression of dominant-negative CREB causes a severe dystrophic phenotype with decreased expression of SIK1 that can be rescued by exogenous SIK1 over-expression (20). In addition, a recent study has demonstrated the importance of Nr4a1 for muscle mass regulation by using both gain- and loss-of-function mouse models (22). These reports suggest that PGC-1α, SIK1, and NR4A1 contribute to muscle hypertrophy induced by Gα-coupled GPCRs. As expected from these studies, Tgr5 KO mice exhibited lower muscle mass with decreased CREB target genes, including SIK1, Nr4a1, and Pgc-1α (Fig. 1, A, C, and D). Since the skeletal muscle reduction rate in Gα KO mice that loses reactivity to all Gα-coupled GPCRs is 20–40% (35), it is a surprising result that Tgr5 KO mice showed about 10% reduction in skeletal muscle mass, suggesting that TGR5 plays an important role as a muscle mass regulatory GPCR (Fig. 1C). Conversely, muscle-specific TGR5 over-expression induces muscle hypertrophy and increases grip strength in young and old mice (Fig. 3, C–G). TLCA-induced TGR5 activation in skeletal muscle promptly raised SIK1, Nr4a1, and Pgc-1α expression, leading to the upregulation of Igf1 expression and the downregulation of Atrogin1 and Musa1 expression (Fig. 3H). Contrary to previous reports (20,21), si SIK1#1 and #2 did not inhibit muscle cell differentiation in C2C12 myoblasts, but rather increase differentiation marker genes in si SIK1#2 (Fig. 2E). It may have been caused by low knockdown efficiency, and/or compensatory mechanisms in the muscle cell to maintain its function. Although our present study could not provide clear answers to this issue, we have been confirmed that TGR5 activation promoted SIK1 expression and TGR5-induced muscle cell differentiation is canceled by SIK1 knockdown (Fig. 2, C–E). These data indicate that SIK1 plays an important role in muscle differentiation by activation of TGR5. Therefore, the decrease in muscle mass observed in TGR5 KO mice is considered to be the result of muscle cell development and growth disorder. Unlike other GPCRs, TGR5 does not interact with β-arrestin, thus it does not desensitize, resulting in a long-lasting activation of cAMP signaling (36). This fact may explain the reason why Tgr5 KO mice exhibited significantly lower muscle mass and strength compared to WT mice in this study, despite endogenous Tgr5 showing just a weak immediate response to single TLCA injection in mouse skeletal muscle (Fig. 1, C and D, and Fig. 3H). Previously, we and others have identified several compounds as TGR5 ligands, and confirmed that these compounds contribute to metabolic improvement (6,37-41). Additionally, obacunone and ursolic acid, both of which are known to activate TGR5, have been shown to increase muscle mass in mice (42,43). Although whether TGR5 is needed for this response has yet to be definitively determined, these results support our notion that TGR5 activation leads to muscle hypertrophy. By using RNA-sequencing data, we found a positive correlation between TGR5 and IGF1 expression levels in human skeletal muscle, which is in agreement with the fact that IGF1 mRNA expression is increased by TGR5 activation in human skeletal muscle myotubes (Fig. 4 and Fig. S3). These results suggest that TGR5 has the potential to increase muscle mass in humans as well. Additional studies are needed to confirm whether TGR5 regulates human skeletal muscle functions.

Regarding TGR5 activity, the TGR5 expression level is an important factor for its downstream activation. For example, Tgr5 mRNA expression is strongly correlated with that of several estimated Tgr5 target genes,
such as CoxVIA1 and ATP synthase subunits, in mouse liver (6). This is supported by our finding that TGR5 mRNA expression is positively correlated with that of IGF1 in human skeletal muscle (Fig. S3). However, the expression mechanism is not well understood. Notably, our study clearly showed that Tgr5 expression is regulated by the UPR in skeletal muscle cells (Fig. 5, A–D). mTgr5 promoter activity was upregulated by both XBP-1 and ATF6α, which recognize combined ERSE-like and UPRE-like elements in the 5′ upstream region of the mTgr5 promoter (Fig. 6, A and B, and Fig. S5). Endogenous Tgr5 upregulation by ATF6α(N) over-expression resulted in a strong response to TLCA in C2C12 cells (Fig. 6E). In accordance with previous reports (16,17), treadmill exercise activated the UPR in mouse skeletal muscle, and we found that exercise increased Tgr5 expression in an Atf6α-dependent manner (Figure 6G). These facts suggest that Atf6α-dependent Tgr5 upregulation in skeletal muscle contributes to exercise-induced muscle hypertrophy. However, contrary to our expectation, basal Tgr5 expression level was increased in Atf6α KO mice. This data indicates that the contribution of Atf6α to resting muscle Tgr5 expression is small and suggests the presence of unknown regulation mechanism of Tgr5 expression. Because TGR5 is known to exert several adverse effects, such as gallstone formation and bile acid-induced itch and analgesia (19,44), other than those beneficial on systemic metabolism and muscle function, tissue-specific regulation of TGR5 expression may be a valid approach to obtain benefit from TGR5 with fewer side effects.

In the postprandial state, farnesoid X receptor is activated by bile acids in ileal enterocytes to promote fibroblast growth factor (FGF) 15/19 secretion, which regulates bile acid metabolism and metabolic homeostasis (45). Interestingly, a recent study shows that FGF19 treatment induces skeletal muscle hypertrophy and ameliorates glucocorticoid, obesity, and aging-induced skeletal muscle atrophy in mice (46). Bile acids may be more closely related to skeletal muscle function than we expected.

In summary, we showed that TGR5 activation in skeletal muscles promotes muscle hypertrophy and differentiation. Moreover, we found that Tgr5 expression was regulated by the UPR in muscle cells, and exercise increased Tgr5 mRNA expression in an Atf6α-dependent manner in mouse skeletal muscle, suggesting a synergistic effect between feeding and exercise on Tgr5 activity by upregulating both blood bile acid concentration and Tgr5 expression (Fig. 7). These results establish a new linkage between bile acid function and skeletal muscle metabolic adjustment, and indicate that muscle TGR5 may be a feasible target for maintaining muscle function in the elderly.

Experimental Procedures

Animals and diets—For a generation of skeletal muscle-specific TGR5 transgenic mice, 3xFlag hTGR5 was cloned into MCK promoter plasmids, gifted from Ronald Kahn (Addgene plasmid # 12528) (47); purified transgene was then injected into C57BL/6 oocytes. Atf6α KO mice were generated as described previously (48). Mice were housed with a 12:12-h light–dark cycle and given free access to water and food. All animal experiments were performed according to the Guideline for the Care and Use of Laboratory Animals of the University of Tokyo, under the approval of the Animal Usage Committee of University of Tokyo (approval numbers P13-812, P15-079 and P17-120).

Treadmill exercise —8–10 weeks old male Atf6α KO mice and WT littermates were acclimated to running on a treadmill (Muromachi kikai) at a 10% incline for 60 min. Running speed was set at 5 m/min for the first 5 min and increased to 10 m/min for the next 5 min. Subsequently, speed was increased to 15 m/min for 20 min and finally 30 min was set at 18 m/min. Treadmill running was continued until the mice were exhausted (they remained on an electric stimulus grid for 6 s) or completed a 60-min running program.

Cell culture—C2C12 myoblasts were cultured in growth medium (DMEM supplemented with 10% FBS). For differentiation to myotubes, the medium was change into
differentiation medium (DMEM supplemented with 2% horse serum) as previously described (49). Human skeletal muscle myoblasts (HSMM) were obtained from Lonza and were cultured in accordance with the supplier’s instructions. For differentiation to myotubes, the medium was changed into differentiation medium (D-MEM/Ham’s F-12 supplemented with 2% horse serum). C2C12 myoblasts and HSMM were infected with 2.5 × 10^6 pfu/mL adenovirus culture overnight, and the media was refreshed the next morning.

**Immunostaining**—C2C12 cells were washed by PBS and fixed with 4% paraformaldehyde in PBS for 15 min at RT. Cells were then permeabilized with 0.5% Triton X-100 in PBS for 5 min and blocked with 3% bovine serum albumin in PBS. Subsequently, the cells were incubated with anti-MyHC antibody (MF20, R&D systems) for 60 min, then incubated with secondary antibody (Fluorescein (FITC)-AffiniPure Donkey Anti-Mouse IgG, Jackson ImmunoResearch) for 30 min.

**Immunoblotting**—C2C12 cells and mouse tissues were lysed with RIPA buffer (50 mM Tris·HCl, pH 7.4, 1 mM EDTA, 150 mM NaCl, 1% Nonidet P-40, and 0.25% sodium deoxycholate) supplemented with a protease inhibitor cocktail (Nacalai Tesque) and a phosphatase inhibitor cocktail (Sigma-Aldrich). The proteins were subjected to SDS-PAGE and analyzed by immunoblotting. Anti-α-tubulin (H-300) and anti-TGR5 (H-90) antibodies were obtained from Santa Cruz Biotechnology. Anti-BiP, anti-AKT and anti-phospho-AKT antibodies were purchased from Cell Signaling Technology. Anti-FLAG (M2) and anti-β-actin (AC-15) antibodies were acquired from Sigma.

**Quantitative RT-PCR**—Total RNA was extracted using ISOGEN (Nippon Gene) according to the manufacturer’s instructions. Total RNA was reverse-transcribed using a high capacity cDNA reverse transcription kit (Applied Biosystems) and subjected to quantitative RT-PCR analysis (TaqMan probe and SYBR Green) by using a StepOnePlus real time PCR system. The values were normalized to 18S. The primers used for the PCR analysis (SYBR green) are described in Table S2.

**siRNA experiments**—siRNAs for mouse Sik1 (AM16708-156369, -156371) and control (AM4611) (ThermoFisher Scientific) were transfected using Lipofectamine RNAiMAX (Invitrogen) into C2C12 myoblasts (25 nM each) according to the manufacturer’s instructions.

**Physiological measurements**—O2 consumption and CO2 production in 8-week-old male Tgr5 KO mice and WT littermates were measured using a ARCO-2000 Mass Spectrometer (ARCO system) with one mouse per chamber. The environment was maintained at 21 ± 3 °C, with 50 ± 10% relative humidity. The grip strength test was performed using a MK-380M grip strength meter (Muromachi Kikai). The grip strength was measured 7 times for each mouse. The same measurements were repeated 4 days later, and the average of the highest values was used.

**Luciferase assay**—C2C12 myoblasts were plated on 12-well plates at a density of 1 × 10^5 cells/well. Cells were cultured with growth medium overnight and transfected with plasmids by a calcium phosphate method. After 24 h of incubation with growth medium or an additional 2 days of differentiation, the luciferase and β-galactosidase activities were determined.

**Statistical analysis**—All results are expressed as means ± SEM of at least three independent biological replicates. Two-tailed unpaired Student’s t tests or one-way ANOVAs (Tukey’s post hoc test) were used to determine p values. Statistical significance was defined as p < 0.05.
Acknowledgments: The authors would like to thank Dr. Gayla Vassileva (Merck Sharp & Dohme Corp.) for providing Tgr5 KO mice. We also thank Enago (www.enago.jp) for the English language review.

Conflict of interest: The authors declare that they have no conflicts of interest with the contents of this article.

Author Contributions: T.S., K.M., and R.S. were involved in the study conception. T.S., M.S., J.I., and R.S. were involved in the study interpretation. T.S. and R.S. designed the study. T.S. performed most of the experiments and supervised the assistance of A.K., M.M. and S.M. The manuscript was written by T.S. and edited by R.S.
References

1. Wahlstrom, A., Sayin, S. I., Marschall, H. U., and Backhed, F. (2016) Intestinal Crosstalk between Bile Acids and Microbiota and Its Impact on Host Metabolism. *Cell Metab* **24**, 41-50

2. Kawamata, Y., Fujii, R., Hosoya, M., Harada, M., Yoshida, H., Miwa, M., Fukusumi, S., Habata, Y., Itoh, T., Shintani, Y., Hinuma, S., Fujisawa, Y., and Fujino, M. (2003) A G protein-coupled receptor responsive to bile acids. *J Biol Chem* **278**, 9435-9440

3. Maruyama, T., Miyamoto, Y., Nakamura, T., Tanai, Y., Okada, H., Sugiyama, E., Itadani, H., and Tanaka, K. (2002) Identification of membrane-type receptor for bile acids (M-BAR). *Biochem Biophys Res Commun* **298**, 714-719

4. Watanabe, M., Houten, S. M., Mataki, C., Christofidelete, M. A., Kim, B. W., Sato, H., Messaddeq, N., Harney, J. W., Ezaki, O., Kodama, T., Schoonjans, K., Bianco, A. C., and Auwerx, J. (2006) Bile acids induce energy expenditure by promoting intracellular thyroid hormone activation. *Nature* **439**, 484-489

5. Altarejos, J. Y., and Montminy, M. (2011) CREB and the CRTC co-activators: sensors for hormonal and metabolic signals. *Nat Rev Mol Cell Biol* **12**, 141-151

6. Thomas, C., Gioiello, A., Noriega, L., Strehle, A., Oury, J., Rizzo, G., Macchiariulo, A., Yamamoto, H., Mataki, C., Pruzanski, M., Pelliciari, R., Auwerx, J., and Schoonjans, K. (2009) TGR5-mediated bile acid sensing controls glucose homeostasis. *Cell Metab* **10**, 167-177

7. Potthoff, M. J., Potts, A., He, T., Duarte, J. A., Taussig, R., Mangelsdorf, D. J., Kliewer, S. A., and Burgess, S. C. (2013) Colesevelam suppresses hepatic glycogenolysis by TGR5-mediated induction of GLP-1 action in DIO mice. *Am J Physiol Gastrointest Liver Physiol* **304**, G371-380

8. Cao, H., Chen, Z. X., Wang, K., Ning, M. M., Zou, Q. A., Feng, Y., Ye, Y. L., Leng, Y., and Shen, J. H. (2016) Intestinally-targeted TGR5 agonists equipped with quaternary ammonium have an improved hypoglycemic effect and reduced gallbladder filling effect. *Sci Rep* **6**, 28676

9. Katsuma, S., Hirasawa, A., and Tsujimoto, G. (2005) Bile acids promote glucagon-like peptide-1 secretion through TGR5 in a murine enteroendocrine cell line STC-1. *Biochem Biophys Res Commun* **329**, 386-390

10. Broeders, E. P., Nascimento, E. B., Havekes, B., Brans, B., Roumans, K. H., Tailleux, A., Schaart, G., Kouach, M., Charton, J., Deprez, B., Bouvy, N. D., Mottaghy, F., Staels, B., van Marken Lichtenbelt, W. D., and Schrauwen, P. (2015) The Bile Acid Chenodeoxycholic Acid Increases Human Brown Adipose Tissue
Activity. Cell Metab 22, 418-426

11. Worthmann, A., John, C., Ruhlemann, M. C., Baguhl, M., Heinsen, F. A., Schaltenberg, N., Heine, M., Schlein, C., Evangelakos, I., Mineo, C., Fischer, M., Dandri, M., Kremsosr, C., Scheja, L., Franke, A., Shaul, P. W., and Heeren, J. (2017) Cold-induced conversion of cholesterol to bile acids in mice shapes the gut microbiome and promotes adaptive thermogenesis. Nat Med

12. Velazquez-Villegas, L. A., Perino, A., Lemos, V., Zietak, M., Nomura, M., Pols, T. W. H., and Schoonjans, K. (2018) TGR5 signalling promotes mitochondrial fission and beige remodelling of white adipose tissue. Nat Commun 9, 245

13. Lee, J., and Ozcan, U. (2014) Unfolded protein response signaling and metabolic diseases. J Biol Chem 289, 1203-1211

14. Wang, M., and Kaufman, R. J. (2014) The impact of the endoplasmic reticulum protein-folding environment on cancer development. Nat Rev Cancer 14, 581-597

15. Walter, P., and Ron, D. (2011) The unfolded protein response: from stress pathway to homeostatic regulation. Science 334, 1081-1086

16. Kim, H. J., Jamart, C., Deldicque, L., An, G. L., Lee, Y. H., Kim, C. K., Raymackers, J. M., and Francaux, M. (2011) Endoplasmic reticulum stress markers and ubiquitin-proteasome pathway activity in response to a 200-km run. Med Sci Sports Exerc 43, 18-25

17. Wu, J., Ruas, J. L., Estall, J. L., Rasbach, K. A., Choi, J. H., Ye, L., Bostrom, P., Tyra, H. M., Crawford, R. W., Campbell, K. P., Rutkowski, D. T., Kaufman, R. J., and Spiegelman, B. M. (2011) The unfolded protein response mediates adaptation to exercise in skeletal muscle through a PGC-1alpha/ATF6alpha complex. Cell Metab 13, 160-169

18. Bohnert, K. R., Gallot, Y. S., Sato, S., Xiong, G., Hindi, S. M., and Kumar, A. (2016) Inhibition of ER stress and unfolding protein response pathways causes skeletal muscle wasting during cancer cachexia. Faseb j

19. Vassileva, G., Golovko, A., Markowitz, L., Abbondanzo, S. J., Zeng, M., Yang, S., Hoos, L., Tetzloff, G., Levitan, D., Murgolo, N. J., Keane, K., Davis, H. R., Jr., Hedrick, J., and Gustafson, E. L. (2006) Targeted deletion of Gpbar1 protects mice from cholesterol gallstone formation. Biochem J 398, 423-430

20. Berdeaux, R., Goebel, N., Banaszynski, L., Takemori, H., Wandless, T., Shelton, G. D., and Montminy, M. (2007) SIK1 is a class II HDAC kinase that promotes survival of skeletal myocytes. Nat Med 13, 597-603

21. Stewart, R., Akhmedov, D., Robb, C., Leiter, C., and Berdeaux, R. (2013) Regulation of SIK1 abundance and stability is critical for myogenesis. Proc Natl
22. Tontonoz, P., Cortez-Toledo, O., Wroblewski, K., Hong, C., Lim, L., Carranza, R., Conneely, O., Metzger, D., and Chao, L. C. (2015) The orphan nuclear receptor Nur77 is a determinant of myofiber size and muscle mass in mice. *Mol Cell Biol* **35**, 1125-1138

23. Ruas, J. L., White, J. P., Rao, R. R., Kleiner, S., Brannan, K. T., Harrison, B. C., Greene, N. P., Wu, J., Estall, J. L., Irving, B. A., Lanza, I. R., Rasbach, K. A., Okutsu, M., Nair, K. S., Yan, Z., Leinwand, L. A., and Spiegelman, B. M. (2012) A PGC-1alpha isoform induced by resistance training regulates skeletal muscle hypertrophy. *Cell* **151**, 1319-1331

24. Sartori, R., Schirwis, E., Blaauw, B., Bortolanza, S., Zhao, J., Enzo, E., Stantzou, A., Mouisel, E., Toniolo, L., Ferry, A., Stricker, S., Goldberg, A. L., Dupont, S., Piccolo, S., Amthor, H., and Sandri, M. (2013) BMP signaling controls muscle mass. *Nat Genet* **45**, 1309-1318

25. Milan, G., Romanello, V., Pescatore, F., Armani, A., Paik, J. H., Frasson, L., Seydel, A., Zhao, J., Abraham, R., Goldberg, A. L., Blaauw, B., DePinho, R. A., and Sandri, M. (2015) Regulation of autophagy and the ubiquitin-proteasome system by the FoxO transcriptional network during muscle atrophy. *Nat Commun* **6**, 6670

26. Vassileva, G., Hu, W., Hoos, L., Tetzloff, G., Yang, S., Liu, L., Kang, L., Davis, H. R., Hedrick, J. A., Lan, H., Kowalski, T., and Gustafson, E. L. (2010) Gender-dependent effect of Gpbar1 genetic deletion on the metabolic profiles of diet-induced obese mice. *J Endocrinol* **205**, 225-232

27. Potthoff, M. J., and Olson, E. N. (2007) MEF2: a central regulator of diverse developmental programs. *Development* **134**, 4131-4140

28. GTEx-Consortium. (2013) The Genotype-Tissue Expression (GTEx) project. *Nat Genet* **45**, 580-585

29. Yamamoto, K., Yoshida, H., Kokame, K., Kaufman, R. J., and Mori, K. (2004) Differential contributions of ATF6 and XBP1 to the activation of endoplasmic reticulum stress-responsive cis-acting elements ERSE, UPRE and ERSE-II. *J Biochem* **136**, 343-350

30. Rajagopal, S., Kumar, D. P., Mahavadi, S., Bhattacharya, S., Zhou, R., Corvera, C. U., Bunnett, N. W., Grider, J. R., and Murthy, K. S. (2013) Activation of G protein-coupled bile acid receptor, TGR5, induces smooth muscle relaxation via both Epac- and PKA-mediated inhibition of RhoA/Rho kinase pathway. *Am J Physiol Gastrointest Liver Physiol* **304**, G527-535
31. Berdeaux, R., and Stewart, R. (2012) cAMP signaling in skeletal muscle adaptation: hypertrophy, metabolism, and regeneration. *Am J Physiol Endocrinol Metab* **303**, E1-17

32. Ryall, J. G., Schertzer, J. D., and Lynch, G. S. (2007) Attenuation of age-related muscle wasting and weakness in rats after formoterol treatment: therapeutic implications for sarcopenia. *J Gerontol A Biol Sci Med Sci* **62**, 813-823

33. Kim, Y. S., and Sainz, R. D. (1992) Beta-adrenergic agonists and hypertrophy of skeletal muscles. *Life Sci* **50**, 397-407

34. Bruno, N. E., Kelly, K. A., Hawkins, R., Bramah-Lawani, M., Amelio, A. L., Nwachukwu, J. C., Nettles, K. W., and Conkright, M. D. (2014) Creb coactivators direct anabolic responses and enhance performance of skeletal muscle. *Embo j* **33**, 1027-1043

35. Chen, M., Feng, H. Z., Gupta, D., Kelleher, J., Dickerson, K. E., Wang, J., Hunt, D., Jou, W., Gavrilova, O., Jin, J. P., and Weinstein, L. S. (2009) G(s)alpha deficiency in skeletal muscle leads to reduced muscle mass, fiber-type switching, and glucose intolerance without insulin resistance or deficiency. *Am J Physiol Cell Physiol* **296**, C930-940

36. Jensen, D. D., Godfrey, C. B., Niklas, C., Canals, M., Kocan, M., Poole, D. P., Murphy, J. E., Alemi, F., Cottrell, G. S., Korbmacher, C., Lambert, N. A., Bunnett, N. W., and Corvera, C. U. (2013) The bile acid receptor TGR5 does not interact with beta-arrestins or traffic to endosomes but transmits sustained signals from plasma membrane rafts. *J Biol Chem* **288**, 22942-22960

37. Genet, C., Strehle, A., Schmidt, C., Boudjelal, G., Lobstein, A., Schoonjans, K., Souchet, M., Auwerx, J., Saladin, R., and Wagner, A. (2010) Structure-activity relationship study of betulinic acid, a novel and selective TGR5 agonist, and its synthetic derivatives: potential impact in diabetes. *J Med Chem* **53**, 178-190

38. Sato, H., Genet, C., Strehle, A., Thomas, C., Lobstein, A., Wagner, A., Mioskowski, C., Auwerx, J., and Saladin, R. (2007) Anti-hyperglycemic activity of a TGR5 agonist isolated from Olea europaea. *Biochem Biophys Res Commun* **362**, 793-798

39. Ono, E., Inoue, J., Hashidume, T., Shimizu, M., and Sato, R. (2011) Anti-obesity and anti-hyperglycemic effects of the dietary citrus limonoid nomilin in mice fed a high-fat diet. *Biochem Biophys Res Commun* **410**, 677-681

40. Sasaki, T., Mita, M., Ikari, N., Kuboyama, A., Hashimoto, S., Kaneko, T., Ishiguro, M., Shimizu, M., Inoue, J., and Sato, R. (2017) Identification of key amino acid residues in the hTGR5-nomilin interaction and construction of its binding model.
41. Sato, R. (2013) Nomilin as an anti-obesity and anti-hyperglycemic agent. *Vitam Horm* **91**, 425-439

42. Horiba, T., Katsukawa, M., Mita, M., and Sato, R. (2015) Dietary obacunone supplementation stimulates muscle hypertrophy, and suppresses hyperglycemia and obesity through the TGR5 and PPARgamma pathway. *Biochem Biophys Res Commun* **463**, 846-852

43. Kunkel, S. D., Sunega, M., Ebert, S. M., Bongers, K. S., Fox, D. K., Malmberg, S. E., Alipour, F., Shields, R. K., and Adams, C. M. (2011) mRNA expression signatures of human skeletal muscle atrophy identify a natural compound that increases muscle mass. *Cell Metab* **13**, 627-638

44. Alemi, F., Kwon, E., Poole, D. P., Lieu, T., Lyo, V., Cattaruzza, F., Cevikbas, F., Steinhoff, M., Nassini, R., Materazzi, S., Guerrero-Alba, R., Valdez-Morales, E., Cottrell, G. S., Schoonjans, K., Geppetti, P., Vanner, S. J., Bunnett, N. W., and Corvera, C. U. (2013) The TGR5 receptor mediates bile acid-induced itch and analgesia. *J Clin Invest* **123**, 1513-1530

45. Kliewer, S. A., and Mangelsdorf, D. J. (2015) Bile Acids as Hormones: The FXR-FGF15/19 Pathway. *Dig Dis* **33**, 327-331

46. Benoit, B., Meugnier, E., Castelli, M., Chanon, S., Vieille-Marchiset, A., Durand, C., Bendridi, N., Pesenti, S., Monternier, P. A., Durieux, A. C., Freyssenet, D., Rieusset, J., Lefai, E., Vidal, H., and Ruzzin, J. (2017) Fibroblast growth factor 19 regulates skeletal muscle mass and ameliorates muscle wasting in mice. *Nat Med* **23**, 990-996

47. Bruning, J. C., Michael, M. D., Winnay, J. N., Hayashi, T., Horsch, D., Accili, D., Goodyear, L. J., and Kahn, C. R. (1998) A muscle-specific insulin receptor knockout exhibits features of the metabolic syndrome of NIDDM without altering glucose tolerance. *Mol Cell* **2**, 559-569

48. Yamamoto, K., Sato, T., Matsui, T., Sato, M., Okada, T., Yoshida, H., Harada, A., and Mori, K. (2007) Transcriptional induction of mammalian ER quality control proteins is mediated by single or combined action of ATF6alpha and XBP1. *Dev Cell* **13**, 365-376

49. Sasaki, T., Nakata, R., Inoue, H., Shimizu, M., Inoue, J., and Sato, R. (2014) Role of AMPK and PPARgamma1 in exercise-induced lipoprotein lipase in skeletal muscle. *Am J Physiol Endocrinol Metab* **306**, E1085-1092
Footnotes
This study was supported by JSPS KAKENHI (Grant No. JP15H05781 to R.S., No. JP16K18699 to T.S.), research grants from the LOTTE Foundation (to M.S.), the Cross-ministerial Strategic Innovation Promotion Program (No. 14533567 to R.S.), and the Japanese Agency for Medical Research and Development (AMED-CREST, No. 16gm0910008h0001 to R.S.).
Figure 1. The effects of Tgr5 knockout on skeletal muscle. 
A, the soleus was isolated from 8-week-old male Tgr5 KO mice and WT littermates, and the mRNA levels of Tgr5 and CREB target genes were measured (n = 9). B, body weights of 8-week-old male Tgr5 KO mice and WT littermates (n = 10–12). C and D, muscle weight (C) and % of BW (D) (n = 10–12). E, grip strength was measured at 8 weeks of age (n = 30–37). F–I, food intake (F), Energy expenditure (G), RER (H) and Act Count (I) of 8-week-old male Tgr5 KO mice and WT littermates were monitored over 96 h (n = 6–8). Data are means ± SEM. Statistical analysis were done using two-tailed unpaired Student’s t test. * $p < 0.05$
Figure 2. TGR5 enhances muscle cell differentiation in C2C12 myoblasts.
A, CRE reporter assay in empty vector- or TGR5 expression vector-transfected C2C12 myoblasts. TLCA (50 μM) was treated for 5 h (n = 3). B, C2C12 myoblasts infected with adenovirus-expressing TGR5 or LacZ were treated with TLCA (50 μM) for 3 h. Nr4a2 and Sik1 mRNA levels were determined by RT-PCR (n = 3). C and D, C2C12 myoblasts were infected with adenovirus containing LacZ or TGR5 before 36 h of differentiation. These cells were differentiated with or without TLCA (20 μM). Media were refreshed every 24 h. (C) Samples were collected at the indicated time points and mRNA expressions were determined by RT-PCR (n = 3). (D) Cells were immunostained for MyHC after 2 days of differentiation (left panel), and then quantified for the differentiation index as percent nuclei in myosin heavy chain-positive cells (right panel). E, C2C12 myoblasts were transfected with Sik1 siRNA or control siRNA. After 12 h, these cells were infected with adenovirus-expressing TGR5 or LacZ. 36 h later, the cells were differentiated with or without TLCA (20 μM) for 48 h. mRNA levels were determined by RT-PCR. n.s., not significant (n = 3). Data are means ± SEM. Statistical analyses were done using two-tailed unpaired Student’s t test or one-way ANOVA (Tukey’s post hoc test) * p < 0.05, ** p < 0.01.
Figure 3. Skeletal muscle-specific TGR5 Tg mice have muscle hypertrophy. 
A–C, food intake (A), body weight (B), and tissue weight (mg) (C) of 8-week-old male littermates of the indicated genotype (n = 8–10). D, dorsal view of WT and Tg mice. E, hematoxylin and eosin staining of the gastrocnemius muscle. F, cross-sectional area frequency distribution of gastrocnemius and average (n = 4). G, grip strength was measured at 2, 6, 9, 12, 15, 18 and 21 months of age (n = 21–26). H, saline (10% BSA) or TLCA (0.4 mg/100μL, saline with 10% BSA) was administrated to the quadriceps of 18 h fasted Tg and WT littermates by single intramuscular injections (100 μL/20g BW). After 3 h or 8 h, quadriceps were isolated and mRNA levels were measured (n = 6–8). I, Akt phospho- and total protein, and Flag protein were measured by western blot in WT and Tg quadriceps 8 h after TLCA intramuscular injection. Data are means ± SEM. Statistical analyses were done using two-tailed unpaired Student’s t test. * p < 0.05, ** p < 0.01.
Figure 4. The effect of TGR5 activation on HSMM gene expression. Human skeletal muscle myotubes infected with adenovirus-expressing TGR5 or LacZ were treated with TLCA (50 μM) for 3 h or 8 h. mRNA levels were determined by RT-PCR (n = 3). Data are means ± SEM. Statistical analysis were done using one-way ANOVA (Tukey’s post hoc test). * p < 0.05, ** p < 0.01
Figure 5. The UPR increases Tgr5 expression in skeletal muscles.

A and B, C2C12 myotubes were treated with thapsigargin (250 nM) or tunicamycin (2.5 µg/mL) for 9 h. (A) mRNA levels were determined by RT-PCR (n = 3). (B) Immunoblot analysis of protein lysates using the indicated antibodies (n = 3, mixture) and the Tgr5 protein level normalized to α-tubulin (n = 3). C, saline (0.2% DMSO, 150 µL) or thapsigargin (20 µM, saline with 0.2% DMSO, 150 µL) was administrated to the quadriceps by single intramuscular injection. After 12 h, the quadriceps were isolated and mRNA levels were measured (n = 5–6). D, C2C12 myoblasts were transfected with mouse the Tgr5 promoter-luciferase construct (1600 bp). 24 h later, the cells were treated with tunicamycin (2.5 µg/mL) for 12 h and luciferase activity was measured and normalized against β-galactosidase activity (n = 3). Data are means ± SEM. Statistical analyses were done using two-tailed unpaired Student’s t test or one-way ANOVA (Tukey’s post hoc test) * p < 0.05, ** p < 0.01
Figure 6. Exercise increases Tgr5 mRNA expression in an Atf6α-dependent manner.
A, C2C12 myoblasts were transfected with the described expression plasmids and the mouse Tgr5 promoter-luciferase construct. 24 h later, the cells were harvested, and luciferase activity was measured and normalized against β-galactosidase activity (n = 3). B, C2C12 myoblasts were transfected with the indicated reporter constructs in the presence or absence of the Atf6α(N) expression plasmid. 24 h after transfection, luciferase assays were performed and normalized against β-galactosidase. Promoter activities in the absence of Atf6α(N) were set at 1 (n = 3). C and D, C2C12 myotubes were infected with adenovirus-expressing Atf6α(N) or LacZ as a control. (C) mRNA levels were determined by RT-PCR (n = 3). (D) Immunoblot analysis of protein lysates using the indicated antibodies (n = 3, mixture) and the Tgr5 protein level
normalized to α-tubulin (n = 3). E, CRE reporter assay in LacZ or ATF6α(N) adenovirus-infected C2C12 myoblasts. TLCA (100 μM) was treated for 5 h. Luciferase activities in DMSO were set at 1 (n = 3). F, C2C12 myoblasts were transfected with expression plasmids for ATF6α(N) and/or PGC-1α, together with the mouse Tgr5 promoter-luciferase construct (2000 bp). The cells were differentiated for 2 days, harvested, and luciferase activity was measured and normalized against β-galactosidase (n = 3). G, total RNA was isolated from the quadriceps of 8-10 weeks old male Atf6α KO mice and WT littermates 5 h post treadmill running or sedentary control. mRNA levels were measured by RT-PCR (n = 6 per group). Data are means ± SEM. Statistical analyses were done using two-tailed unpaired Student’s t test or one-way ANOVA (Tukey’s post hoc test) * p < 0.05, ** p < 0.01
Figure 7. A proposed model for roles of the Tgr5 in skeletal muscle. 
Tgr5 expression is increased by Atf6α in muscle cells and it is mimicked by exercise in vivo. Tgr5 activation promotes muscle hypertrophy and muscle cell differentiation.
The exercise-inducible bile acid receptor Tgr5 improves skeletal muscle function in mice
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J. Biol. Chem. published online May 17, 2018

Access the most updated version of this article at doi: 10.1074/jbc.RA118.002733

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