# Direct Activation of Glucose Transport in Primary Human Myotubes After Activation of Peroxisome Proliferator–Activated Receptor δ

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**Activators of peroxisome proliferator–activated receptor (PPAR)γ have been studied intensively for their insulin-sensitizing properties and antidiabetic effects. Recently, a specific PPARδ activator (GW501516) was reported to attenuate plasma glucose and insulin levels when administered to genetically obese *ob/ob* mice. This study was performed to determine whether specific activation of PPARδ has direct effects on insulin action in skeletal muscle. Specific activation of PPARδ using two pharmacological agonists (GW501516 and GW0742) increased glucose uptake independently of insulin in differentiated C2C12 myotubes. In cultured primary human skeletal myotubes, GW501516 increased glucose uptake independently of insulin and enhanced subsequent insulin stimulation. PPARδ agonists increased the respective phosphorylation and expression of AMP-activated protein kinase 1.9-fold ($P < 0.05$) and 1.8-fold ($P < 0.05$), of extracellular signal–regulated kinase 1/2 mitogen-activated protein kinase (MAPK) 2.2-fold ($P < 0.05$) and 1.7-fold ($P < 0.05$), and of p38 MAPK 1.2-fold ($P < 0.05$) and 1.4-fold ($P < 0.05$). Basal and insulin-stimulated protein kinase B/Akt was unaltered in cells preexposed to PPARδ agonists. Preincubation of myotubes with the p38 MAPK inhibitor SB203580 reduced insulin- and PPARδ-mediated increase in glucose uptake, whereas the mitogen-activated protein kinase kinase inhibitor PD98059 was without effect. PPARδ agonists reduced mRNA expression of PPARδ, sterol regulatory element binding protein (SREBP)-1a, and SREBP-1c ($P < 0.05$). In contrast, mRNA expression of PPARγ, PPARγ coactivator 1, GLUT1, and GLUT4 was unaltered. Our results provide evidence to suggest that PPARδ agonists increase glucose metabolism and promote gene regulatory responses in cultured human skeletal muscle. Moreover, we provide biological validation of PPARδ as a potential target for antidiabetic therapy.** *Diabetes* **54:1157–1163, 2005**

Type 2 diabetes is a clinical disorder of glucose and lipid metabolism that is partly caused by impaired glucose uptake into adipose tissue and skeletal muscle (1). Although genetic and environmental (dietary and lifestyle) factors contribute to the development of insulin resistance and the onset of type 2 diabetes (2), the exact mechanism for the metabolic changes accompanying these disorders has not yet been completely elucidated. This is partly reflected by the complex interaction between insulin target tissues including adipose tissue, an endocrine organ that secretes several hormones and cytokines, and skeletal muscle, a major site of insulin resistance in type 2 diabetic patients (3,4).

Peroxisome proliferator–activated receptors (PPARs) play key roles in defining adipose tissue mass modulating fat and carbohydrate metabolism and storage (5). Three closely related PPAR subtypes have been described: α, β/δ, and γ (6–8). The different PPAR subtypes display tissue-specific expression; PPARγ is primarily expressed in adipocytes (7,9), PPARα is most abundant in liver, and PPARδ (also referred to as PPARβ) is expressed in a wide variety of tissues, with high levels in skeletal muscle (9). PPARγ plays a key role in adipocyte differentiation and lipid storage (rev. in 7). Thiazolodinediones (TZDs) are a class of insulin-sensitizing drugs that specifically activate PPARγ and promote adipocyte differentiation (10,11). Treatment of type 2 diabetic patients with TZDs improves glucose homeostasis (12,13) and alters gene expression in adipocytes (8). PPARα is activated by fibrates, a class of cholesterol-lowering drugs used in the treatment of lipid disorders, and functions by enhancing β-oxidation in the liver. In contrast to PPARα and PPARγ, pharmacological treatments to activate PPARδ in humans with metabolic disease are lacking.

Activation of PPARδ is linked to oxidative metabolism. The role of PPARδ on whole-body glucose homeostasis has been evaluated in muscle-specific PPARδ transgenic mice (14). PPARδ transgenic mice are characterized by enzymatic and gene expression profiles that promote oxidative metabolism in skeletal muscle. Moreover,

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AICAR, 5-aminoimidazol-4-carboxamide-1β-D-ribonucleoside; AMPK, AMP-activated protein kinase; DMEM, Dulbecco's minimum essential medium; ERK, extracellular signal–regulated kinase; FBS, fetal bovine serum; MAPK, mitogen-activated protein kinase; MEK, mitogen-activated protein kinase kinase; PGC, peroxisome proliferator–activated receptor γ coactivator; PKB, protein kinase B; PPAR, peroxisome proliferator–activated receptor; SREBP, sterol regulatory element binding protein; TZD, thiazolodinedione.

PPARδ transgenic mice have reduced body fat mass due to a reduction of adipose cell size (15). Specific activation of PPARδ using a pharmacological activator, GW501516 (16,17), has been reported to have positive effects on expression of genes involved in cardiac lipid metabolism in neonatal rat cardiomyocytes (18). When administered to insulin-resistant middle-aged obese rhesus monkeys, GW501516 causes a dramatic dose-dependent rise in serum HDL cholesterol, while lowering the levels of small dense LDL and fasting triglycerides and reducing fasting insulin (16). Short-term treatment of obese mice with GW501516 causes dramatic lipid depletion in tissues (15) and attenuates plasma glucose and insulin levels (19).

Given the importance of skeletal muscle insulin resistance in the development of type 2 diabetes and other metabolic disease, we hypothesized that targeted activation of PPARδ in skeletal muscle could have direct effects on glucose metabolism by improving insulin action. We determined whether direct activation of PPARδ using pharmacological activators alters glucose metabolism, signal transduction, and gene expression in cultured primary human muscle cells. We also elucidated whether similar effects are noted in cultured mouse muscle cell (C2C12) and adipocyte cell (3T3-L1) lines.

## RESEARCH DESIGN AND METHODS

Dulbecco's minimum essential medium (DMEM), Ham's F-10 medium, fetal bovine serum (FBS), penicillin, streptomycin, and fungizone were obtained from GibcoBRL Life Technologies (Stockholm, Sweden). Radiochemicals, 2-[G-$^3$H]deoxy-D-glucose (6.0 Ci · mmol$^{-1}$ · l$^{-1}$), and D-[U-$^{14}$C]glucose (310 mCi · mmol$^{-1}$ · l$^{-1}$), were from Amersham (Uppsala, Sweden). All other chemicals were analytical grade and from Sigma-Aldrich Sweden AB (Stockholm, Sweden). Phosphospecific antibodies against protein kinase B (PKB) (Ser$^{473}$) and extracellular signal–related kinase (ERK)1/2 mitogen-activated protein kinase (MAPK) (Thr$^{202}$ and Tyr$^{204}$) were from New England Biolabs (Beverly, MA), and AMP-activated protein kinase (AMPK) pan α-subunit (Thr$^{172}$) and p38 MAPK (Thr$^{180}$ and Tyr$^{182}$) were from Cell Signaling Technology (Beverly, MA). Antibodies against ERK1/2 were from Transduction Laboratories (Lexington, KY), p38 MAPK and PKB/Akt were from New England Biolabs, AMPKα1 and α2 were from Upstate (Lake Placid, NY); and GLUT1 and GLUT4 were from Biogenesis (Poole, UK) and Genzyme (Cambridge, MA), respectively. Horseradish peroxidase–conjugated goat anti-rabbit and anti-mouse immunoglobulin G were from Bio-Rad Laboratories (Richmond, CA). Reagents for enhanced chemiluminescence were from Amersham (Arlington Heights, IL). GW501516 was synthesized by Synthelec AB (Lund, Sweden), and selectivity of this compound has been described (16). GW0742 was a gift from GlaxoSmithKline (17). GW501516 and GW0742 were dissolved in sterile DMSO. In every experiment performed, the DMSO content for baseline condition was adjusted to the amount of DMSO added with the agonist. PD98059 and SB203580 were purchased from Sigma-Aldrich Sweden AB and dissolved in sterile DMSO.

**Human primary skeletal muscle cell cultures.** Skeletal muscle biopsies were obtained from healthy individuals who underwent general surgery. None of the subjects had known metabolic disease. Satellite cells were isolated, and primary muscle cultures were established (20). The ethical committee at Karolinska Institute approved protocols. Cells were grown in DMEM (1,000 mg/l glucose) with 10% FBS and 1% penicillin/streptomycin in noncoated dishes. To differentiate human myoblasts into myotubes, dishes with a cell density of 80–90% were grown in DMEM with 4% FBS for 2 days to induce myotube formation and then grown in DMEM with 2% FBS for 2 days. Before use, the cells were controlled optically for formation of elongated myotubes and serum starved overnight.

**3T3L1 cell cultures.** Mouse 3T3L1 fibroblasts were grown in DMEM (4,500 mg/l glucose) with 10% FBS and 1% penicillin/streptomycin. Cells were cultured until 80–90% confluency for passage. Differentiation was initiated at day 2 after 100% confluency. For initiation of differentiation, 0.25 μmol/l dexamethasone, 0.5 mmol/l isobutylmethylxanthine, and 167 nmol/l insulin were added to DMEM. After 72 h of cultivation, medium was changed to DMEM containing 167 nmol/l insulin. After 2 more days, medium was switched to the initial culturing medium of DMEM (4,500 mg/l glucose) with 10% FBS and 1% penicillin/streptomycin. The cells were used ~12 days after completion of the differentiation protocol, when >90% of the cells expressed the adipocyte phenotype (i.e., filled with fat droplets). Before experimentation, the cells were washed and preincubated with DMEM containing 5 mmol/l glucose, 25 mmol/l HEPES (pH 7.4), and 1% penicillin/streptomycin without FBS.

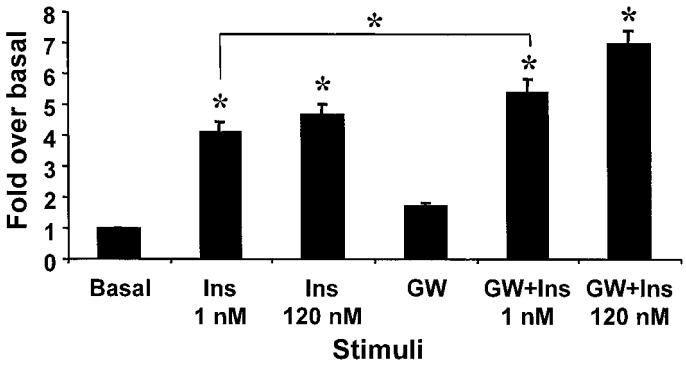


**FIG. 1. Glucose uptake in 3T3-L1 mouse adipocytes incubated for 6 h with or without 10 nmol/l GW501516 (GW). *$P$ < 0.01 vs. basal or vs. 1 nmol/l insulin–treated cells as indicated ($n$ = 9). Insulin stimulation was included for the last 60 min.**

**C2C12 cell cultures.** Mouse C2C12 myoblasts were cultured in DMEM (1,000 mg/l glucose) containing 20% FBS. For initiation of differentiation, the medium was changed to a 2% FBS content when cells were 100% confluent. The cells were grown until days 5–7 for formation of myotubes and then used for experimentation.

**Glucose uptake.** Glucose uptake was performed as previously described for primary human muscle cells (21) and 3T3-L1 adipocytes (22), respectively. When inhibition was performed before glucose uptake experiments, cells were preincubated with ERK1/2 and p38 MAPK inhibitors PD98059 (50 μmol/l) or SB203580 (10 μmol/l). The inhibitors were added to Krebs buffer with either GW0742 in DMSO or DMSO alone (as basal) for incubation at 37°C for 15 min, and insulin was added as indicated.

**Western blot analysis.** Cell monolayers were washed once in ice-cold PBS and harvested directly by scraping into ice-cold lysis buffer (135 mmol/l NaCl, 1 mmol/l $MgCl_2$, 2.7 mmol/l KCl, 20 mmol/l Tris [pH 8.0], 0.5 mmol/l $Na_3VO_4$, 10 mmol/l NaF, 1% Triton X-100, 10% [vol/vol] glycerol, 0.2 mmol/l phenylmethysulfonyl, 10 μg/ml leupeptin, 10 μg/ml antipain, and 10 μg/ml aprotinin). Lysates (20 μg) were separated by SDS-PAGE, transferred to Immobilon-P membranes (Millipore), and probed with primary phosphospecific or protein-specific antibodies and secondary horseradish peroxidase–conjugated antibodies. Proteins were visualized by enhanced chemiluminescence and quantified by densitometry. To correct for loading, the lower part of the each membrane was blotted for histone H3.

**Analysis of mRNA expression.** Quantitative PCR was performed as described previously (21). Primers and probes are available on request.

**Statistics.** Data are presented as means ± SE. Statistical differences were determined by ANOVA multiple comparison using Fisher's least significant differences test or Student's $t$ test, as appropriate. Significant differences were accepted at $P < 0.05$.

## RESULTS

**Effect of a PPARδ agonist on glucose uptake in 3T3-L1 adipocytes.** Activation of PPARδ in adipose tissue decreases fat mass and lipid accumulation (15). To determine whether a PPARδ agonist has direct effects on glucose uptake, differentiated 3T3-L1 adipocytes were incubated with the PPARδ activator GW501516 and/or insulin (Fig. 1). Cultures were incubated in the absence or presence of 10 nmol/l GW501516 for 6 h, followed by addition of [$^3$H]2-deoxyglucose for a further 10 min. Incubation of 3T3-L1 adipocytes with GW501516 was without significant effect on basal glucose uptake (basal uptake was 13.1 pmol glucose · min$^{-1}$ · mg$^{-1}$, and uptake rate with GW501516 was 22.3 pmol glucose · min$^{-1}$ · mg$^{-1}$). Submaximal (1 nmol/l) and maximal (120 nmol/l) insulin-

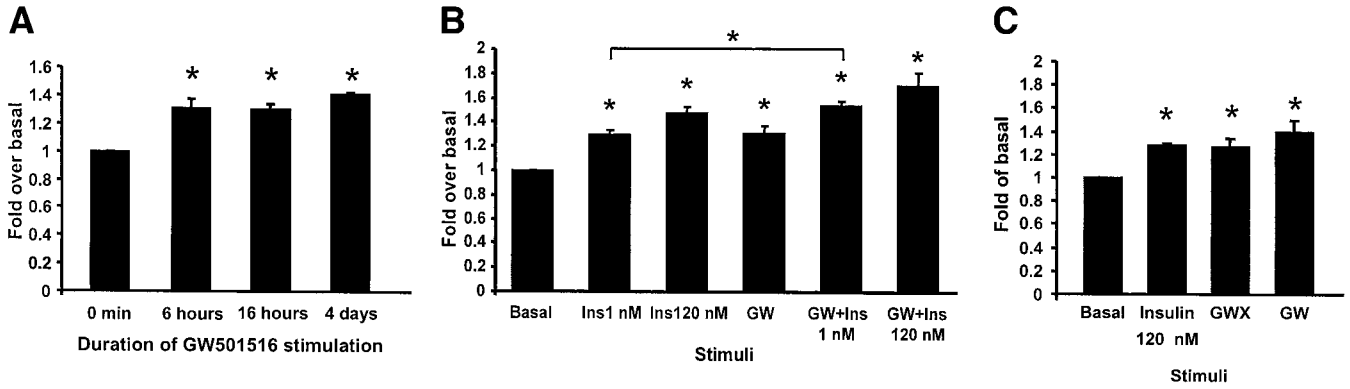


**FIG. 2.** ***A*****: Glucose uptake in primary human myotubes after exposure to 10 nmol/l GW501516 for 6 h, 16 h, and 4 days (*$P < 0.01$. vs. basal; $n$ = 3–7).** ***B*****: Glucose uptake in primary human myotubes after 6 h of treatment with 10 nmol/l GW501516 (*$P < 0.01$ vs. basal or vs. 1 nmol/l insulin–treated cells as indicated; $n = 7$). Insulin stimulation was included for the last 60 min.** ***C*****: Glucose uptake in C2C12 myotubes after 16 h of treatment with either 10 nmol/l GW501516 or 10 nmol/l GW0742. *$P < 0.05$ vs. basal ($n$ = 4–6)**

stimulated glucose uptake was enhanced ($P < 0.01$). The glucose uptake rate with 1 nmol/l insulin alone was 53.4 pmol · $min^{-1}$ · $mg^{-1}$, and in combination with GW501516 treatment, it was 70.6 pmol glucose · $min^{-1}$ · $mg^{-1}$. The glucose uptake rate with 120 nmol/l insulin increased from 61.1 to 90.8 pmol glucose · $min^{-1}$ · $mg^{-1}$ after exposure to GW501516.

**Effect of a PPARδ agonist on glucose uptake in cultured human and mouse myotubes.** We next determined whether a PPARδ agonist has direct effects on glucose uptake in cultured differentiated human skeletal muscle. We established a time course for PPARδ-mediated effects on glucose uptake (Fig. 2*A*). Myotubes were treated with 10 nmol/l GW501516 for 6 h, 16 h, or 4 days. At all time points, GW501516 led to a similar 1.3- to 1.4-fold increase in [$^3$H]2-deoxyglucose uptake ($P < 0.01$). To assess whether PPARδ agonists alter insulin-stimulated glucose uptake in cultured human skeletal muscle cells, cultures were incubated in the absence or presence of 10 nmol/l GW501516 for 6 h, followed by addition of [$^3$H]2-deoxyglucose for a further 10 min. Insulin exposure (1 and 120 nmol/l for 1 h) led to modest 1.3- and 1.5-fold increases in glucose transport (average basal glucose-uptake rate was 3.6 vs. 5.3 pmol glucose · $min^{-1}$ · $mg^{-1}$ for basal versus insulin [120 nmol/l]-stimulated conditions, respectively; $P < 0.01$), and combined exposure of myotubes to GW501516 and insulin resulted in a partial additive effect on glucose uptake (Fig. 2*B*). Finally, we assessed whether the PPARδ agonist would result in a similar effect on glucose uptake in a mouse muscle cell line. Differentiated C2C12 myotubes were exposed to either 10 nmol/l GW501516 or 10 nmol/l GW0742 for 16 h. Exposure of cells to either agonist resulted in a modest but significant increase in glucose uptake (1.4-fold [$P < 0.05$] and 1.3-fold [$P < 0.05$] over basal, respectively) (Fig. 2*C*). Thus, PPARδ agonists enhance glucose uptake in cultured primary human skeletal muscle, as well as mouse adipose and skeletal muscle cells lines.

**Effect of a PPARδ agonist on the expression of candidate genes in primary human myotubes.** Activation of PPARs regulates gene expression in target tissues (rev. in 23). Thus, we hypothesized that exposure of muscle cells to a PPARδ agonist would lead to changes gene expression. mRNA was isolated from primary human muscle cells incubated for 6 h in the absence or presence of GW501516; and expression of PPARδ, PPARγ, PPARγ coactivator (PGC)1, GLUT1, GLUT4, SREBP-1a, and SREBP-1c was determined using quantitative real-time PCR. mRNA expression of PPARγ, PGC1, GLUT1, and GLUT4 was unchanged after exposure to GW501516 (Fig. 3*A–C*). However, mRNA expression of PPARδ, SREBP-1a, and SREBP-1c was repressed (Fig. 3*B* and *C*, $P < 0.05$).

**Effects of a PPARδ agonist on protein expression and phosphorylation in primary human myotubes.** To determine the possible molecular mechanisms for the observed changes in [$^3$H]2-deoxyglucose uptake, differentiated muscle cultures were treated with GW501516 and insulin, as described above for glucose uptake, and protein expression

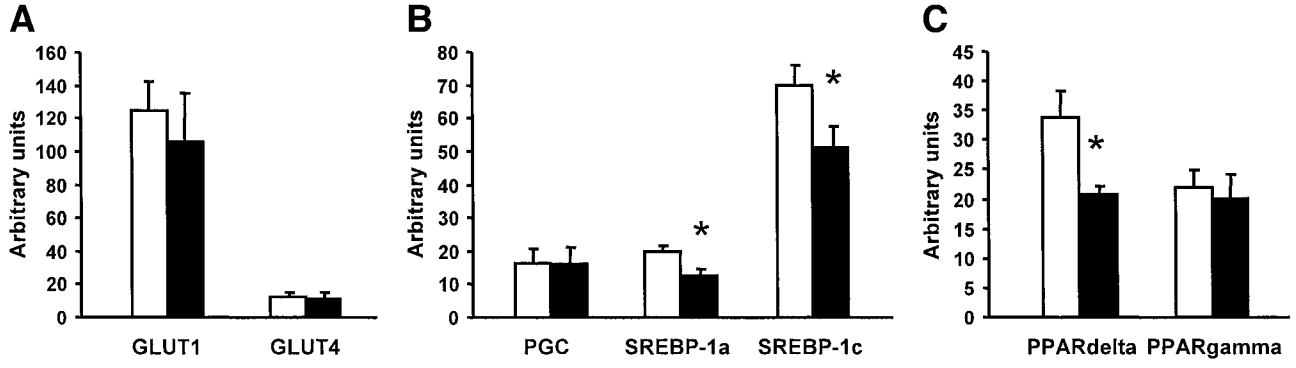


**FIG. 3. Gene expression in primary human myotubes as assessed by quantitative PCR. Cells were incubated for 6 h with (■) or without (□) GW501516. *$P < 0.05$ vs. unstimulated cells ($n$ = 4–6).**

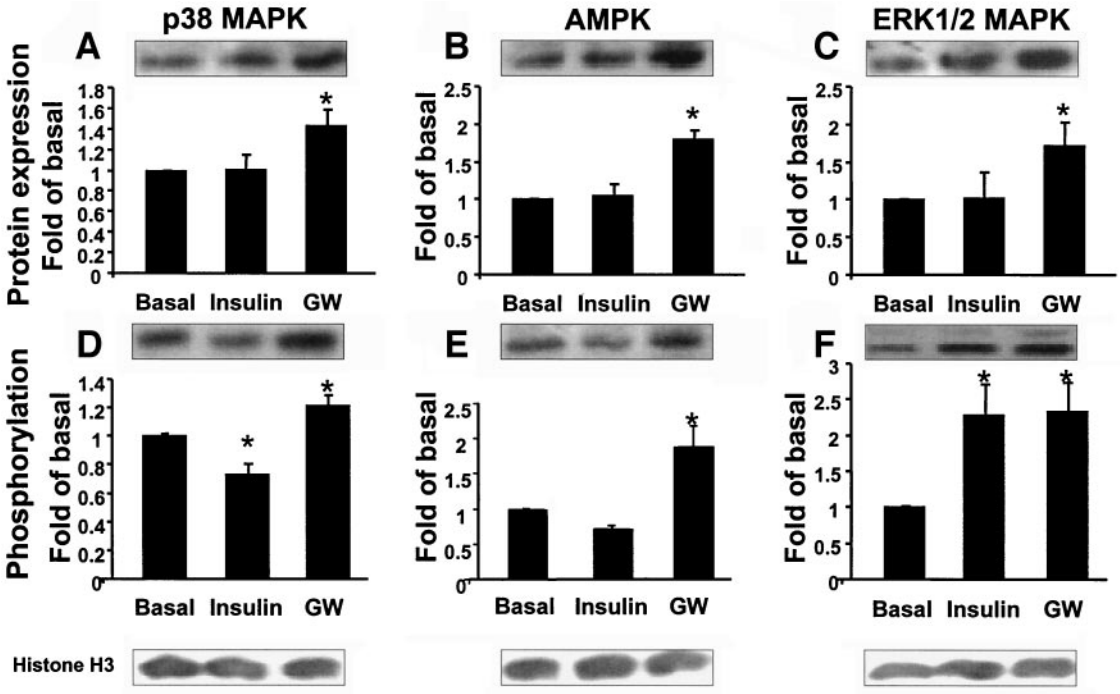


**FIG. 4. Western blotting analysis of phosphorylation and expression of p38 MAPK (*A* and *D*), AMPKα2 (*B* and *E*), and ERK1/2 MAPK (*C* and *F*) after 6 h of exposure to 10 nmol/l GW501516 or 30 min of exposure to 120 nmol/l insulin in primary human muscle cells. Inset shows a representative Western blot; graphs show summarized data ($n$ = 5–8) with means ± SE (*$P$ < 0.05 vs. basal). Histone panel shows even loading.**

and phosphorylation were assessed. Because effects of the PPARδ agonist on insulin-mediated glucose uptake were partly additive, we determined phosphorylation of AMPK, a recently identified member of a putative insulin-independent signaling pathway that has been shown to mediate glucose transport in response to cellular stress or 5-aminoimidazol-4-carboxamide-1β-D-ribonucleoside (AICAR) (a synthetic AMPK activator) (23). Treatment of differentiated human myotubes with GW501516 led to a 1.8-fold increase in expression of AMPKα2 (similar results were seen for AMPKα1; data not shown) and a 1.9-fold increased phosphorylation of AMPK ($P < 0.05$). In contrast, insulin was without effect on either AMPK expression or phosphorylation (Fig. 4*B*). Insulin and cellular stress activate MAPK signaling (24,25). Thus, we determined whether the PPARδ agonist increased either ERK1/2 (Fig. 4*C*) or p38 MAPK (Fig. 4*A*) expression and phosphorylation. Similar to results for AMPK, exposure of myotubes to the PPARδ agonist increased ERK1/2 expression (1.7-fold, $P < 0.05$) and phosphorylation (2.2-fold, $P < 0.05$). Expression of p38 MAPK increased 1.4-fold ($P < 0.05$), and phosphorylation increased 1.2-fold ($P < 0.05$). To determine whether the PPARδ agonist increased components of the insulin signaling pathway directly implicated in glucose transport, we assessed effects on PKB/Akt phosphorylation (Fig. 4). Insulin increased PKB/Akt phosphorylation 4.2-fold ($P < 0.05$). In contrast, GW501516 was without effect on basal phosphorylation or insulin-stimulated PKB/Akt phosphorylation (Fig. 5). Furthermore, expression of GLUT4 and GLUT1 was unaltered (data not shown), confirming the mRNA profile data obtained using quantitative PCR.

**Effect of MEK (PD98059) and p38 MAPK (SB203580) inhibitors on PPARδ-mediated glucose uptake in primary human myotubes.** To determine whether the increased phosphorylation of ERK1/2 and p38 MAPK has functional consequences for PPARδ-mediated effects on glucose uptake, we performed glucose uptake experiments following the protocol described above after a 15-min preincubation with ERK1/2 and p38 MAPK inhibitors (50 μmol/l PD98059 or 10 μmol/l SB203580, respectively). Incubation of cells with the MEK inhibitor PD98059 was without effect on either insulin- or GW501516-mediated glucose uptake (Fig. 6). In contrast, preexposure of cells to the p38 MAPK inhibitor SB203580 reduced insulin-stimulated glucose uptake by 67% ($P < 0.05$) and similarly blunted (65% reduction) the GW501516-stimulated glucose uptake ($P < 0.05$). The additive effect of GW501516 and

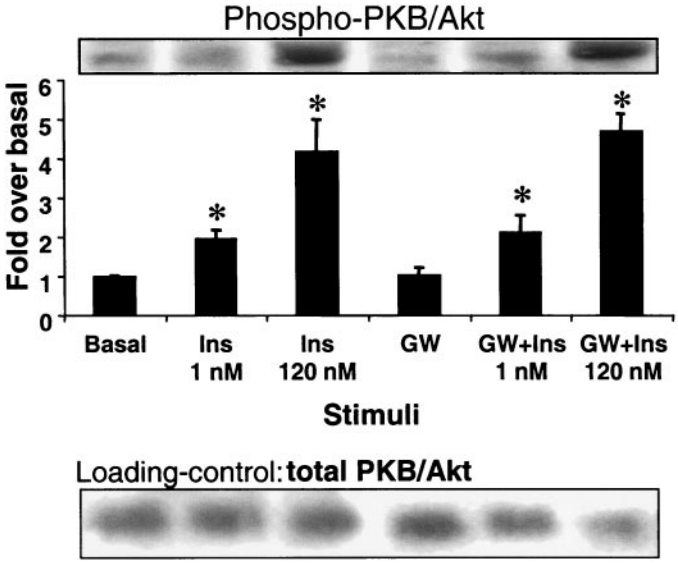


**FIG. 5. PKB phosphorylation in primary human myotubes after a 6-h exposure to GW501516 (GW). *$P$ < 0.05 vs. unstimulated cells ($n$ = 8–9). Cells were stimulated with insulin for 30 min. Lower panel shows even loading of PKB.**

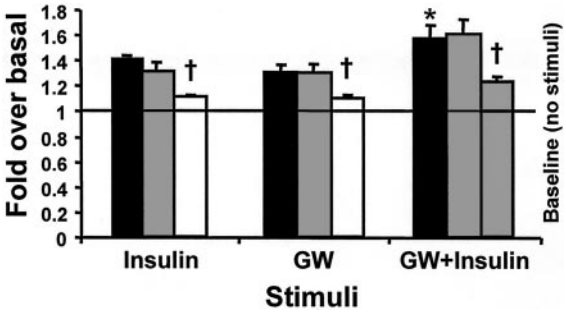


**FIG. 6. Glucose uptake in differentiated primary human myotubes exposed to 16 h with GW501516 (GW) and preincubated without (■) or with (▨, MEK1 inhibitor) 50 μmol/l PD98059 or 10 μmol/l SB203580 (□, p38 MAPK inhibitor) (added 15 min before GW501516). †□ vs. ■, $P < 0.05$. *GW0742 treated vs. GW0742 plus insulin. Insulin (120 nmol/l) was added 60 min before the end of the experiment. Data shown are fold over basal (means ± SE for four separate experiments).**

insulin (120 nmol/l) on glucose uptake was also reduced after exposure to the p38 MAPK inhibitor ($P < 0.05$). Thus, the p38 MAPK inhibitor attenuates insulin and PPARδ agonist–mediated glucose uptake.

## DISCUSSION

Recent work has highlighted a potential role for PPARδ in regulation of fatty acid metabolism in adipose tissue and skeletal muscle (14,16,18,19,26). Here, we provide novel evidence that a PPARδ agonist directly increases skeletal muscle glucose uptake in both cultured primary human muscle cells and mouse C2C12 muscle cells. In differentiated primary human skeletal muscle cultures, insulin sensitivity on glucose uptake was also increased after exposure to a PPARδ agonist. An insulin-sensitizing effect of PPARδ activation on glucose uptake was also noted in differentiated 3T3-L1 adipocyte cells. Furthermore, the effect of the PPARδ agonist on glucose uptake in differentiated primary human myotubes is coincident with increased expression and phosphorylation of ERK1/2, p38 MAPK, and AMPK. Thus, in addition to previously reported effects on gene expression, GW501516 directly enhances glucose uptake through an insulin-independent mechanism.

PPARγ and PPARα have been recognized as important regulators of lipid and carbohydrate metabolism and are targets for pharmacological compounds such as glitazones and fibrates, respectively (23). The role of PPARδ has been less well defined. Improvements in cholesterol profiles and fasting insulin have been observed in insulin-resistant middle-aged obese rhesus monkeys after treatment with GW501516 (16). Moreover, targeted activation of PPARδ in adipose tissue in mice induces genes required for fatty acid oxidation and energy dissipation and protects mice against diet-induced obesity and hyperlipidemia (15). Thus, PPARδ is emerging as a key therapeutic target for regulation of fatty acid metabolism. Here, we provide evidence that in addition to fatty acid metabolism, PPARδ agonists enhance glucose transport. Our results suggest that the insulin-sensitizing effects of PPARδ activation noted in both primate (16) and rodent (15) models probably reflect enhanced whole-body lipid profiles, as well as improvements in insulin action on glucose uptake in adipose tissue and skeletal muscle. Therefore, activation of PPARδ has beneficial effects on fatty acid metabolism, presumably through promoting changes in gene expression, as well as direct effects on glucose metabolism and insulin sensitivity. Targeted expression of PPARδ in transgenic mice resulted in an increased proportion of type 1 (slow, oxidative) muscle fibers (24). An increased proportion of type 1 skeletal muscle has been linked to protection against diet-induced insulin resistance (25). Thus, PPARδ is a highly attractive target for the treatment of metabolic disease.

In an effort to resolve the possible signaling mechanism by which PPARδ agonists increase glucose uptake, we determined phosphorylation of several signaling molecules known to regulate glucose metabolism. Expression and phosphorylation of ERK1/2, p38 MAPK, and AMPK increased in human myotubes in response to the PPARδ agonist. ERK1/2 is unlikely to be involved in glucose uptake (27), and this signaling pathway has been implicated in gene regulatory responses in cultured myotubes (28). Thus, unsurprisingly, preincubation of cells with the MEK inhibitor PD98059 resulted in reduced ERK phosphorylation but did not affect glucose uptake. In contrast, inhibition of p38 MAPK partially reduced glucose uptake in response to either insulin or the PPARδ agonist. p38 MAPK has been directly implicated in glucose uptake (4), although the role of p38 MAPK signaling in glucose uptake has been debated (29). p38 MAPK has also been proposed to be a downstream target of AMPK and a required component for AICAR-mediated AMPK signaling to glucose uptake in Clone 9 cells (30). We provided evidence of a significant reduction in both insulin- and PPARδ-mediated glucose uptake after inhibition of p38 MAPK using SB203580. This observation would lend support to the involvement of the p38 MAPK signaling pathway in mediating insulin, as well as PPARδ-mediated glucose uptake in primary human myotubes. SB203580 has been reported to have a small inhibitory effect on PKB/Akt activity (31). However, our results exclude a role for PKB/Akt signaling in glucose transport in response to PPARδ activation, because no effect was noted on either basal or insulin-stimulated PKB/Akt phosphorylation. Thus, the PKB/Akt pathway appears to be an unlikely candidate for mediating glucose uptake in skeletal muscle in response to the PPARδ agonist.

We provide evidence that PPARδ agonists increase AMPK phosphorylation in human skeletal muscle cultures. AMPK is activated in skeletal muscle in response to increased intracellular AMP concentrations and has been proposed to serve as a metabolic fuel sensor (32). AMPK is also a potent regulator of lipid metabolism, protein synthesis, glucose metabolism, and insulin-independent signals known to enhance glucose uptake in skeletal muscle (rev. in 33). Activation of AMPK has been observed in adult skeletal muscle after physical exercise (34) and in cultured cells in response to incubation with metformin and rosiglitazone (35). AMPK activation with the synthetic AMPK activator, AICAR, leads to an insulin-sensitizing effect in isolated rat epitrochlearis muscle (36) and skeletal muscle from type 2 diabetic patients (37), underscoring the possible role of AMPK in the insulin-sensitizing effect of GW501516 noted in cultured human myotubes reported in this study.

We determined whether the enhanced insulin-mediated

glucose disposal elicited by PPARδ agonists was associated with changes in glucose transporter gene expression in cultured myotubes. mRNA and protein expression of GLUT1 and GLUT4 was unaltered in myotubes exposed to GW501516, consistent with a previous report in C2C12 mouse muscle cells (26). Thus, the PPARδ agonist increases glucose uptake without modifying the total amount of glucose transporters. Therefore, activation of AMPK and/or p38 MAPK, rather than changes in glucose transporter expression, offer an attractive hypothesis for the direct effects of PPARδ activation on glucose uptake in human myotubes.

We also determined whether the PPARδ agonist promoted changes in expression of genes involved in lipid homeostasis. PPARδ, SREBP-1a, and SREBP-1c expression was repressed in response to GW501516 treatment. SREBPs and PPARs are involved in lipid homeostasis, but exhibit opposing actions, with SREBPs controlling genes causing biosynthesis of cholesterol, fatty acids, triacylglycerols, and phospholipids (rev. in 38) and PPARs controlling fatty acid oxidation and lipid storage (rev. in 7). SREBP1-c expression is reduced in response to AMPK activation in 3T3-L1 adipocytes (39). Our finding of reduced GW5051516-mediated expression of SREBPs in skeletal muscle contrasts with a previous report on rodent adipose tissue, in which activation of PPARδ was without effect on SREBP expression (19). Whether this discrepancy is due to species- or tissue-specific differences or to variations in the time of PPARδ activation is unknown. However, the reduction in PPARδ expression in myotubes after GW5051516 exposure is comparable with the reported TZD-mediated downregulation of PPARγ expression in human embryonic kidney 293 cell lines (40), 3T3-L1 rat adipocytes (41), and human skeletal muscle (42) and is consistent with the notion that prolonged exposure to PPAR agonists downregulates nuclear receptor expression.

In conclusion, we provide evidence that exposure of cultured primary human skeletal muscle and C2C12 mouse muscle to specific PPARδ agonists enhances basal and insulin-stimulated glucose uptake. Activation of PPARδ in 3T3-L1 adipocytes enhances insulin-stimulated glucose uptake. In cultured primary human skeletal muscle, PPARδ agonists increase glucose uptake in an insulin-independent manner and lead to the phosphorylation of AMPK and p38 MAPK. Our results underscore the potential therapeutic usefulness of PPARδ agonists for the treatment of metabolic disorders.

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