Am J Physiol Regul Integr Comp Physiol 299: R470–R479, 2010.
First published June 10, 2010; doi:10.1152/ajpregu.00431.2009.

Oral administration of a PPAR-δ agonist to rodents worsens, not improves, maximal insulin-stimulated glucose transport in skeletal muscle of different fibers

Justin Cresser,[1] Arend Bonen,[1] Adrian Chabowski,[2] Leslie E. Stefanyk,[1] Roberto Gulli,[1] Ian Ritchie,[1] and David J. Dyck[1]

[1]*Department of Human Health and Nutritional Sciences, University of Guelph, Guelph, Ontario, Canada; and* [2]*Department of Physiology, Medical University of Bialystok, Bialystok, Poland*

Submitted 20 July 2009; accepted in final form 3 June 2010

Cresser J, Bonen A, Chabowski A, Stefanyk LE, Gulli R, Ritchie I, Dyck DJ. Oral administration of a PPAR-δ agonist to rodents worsens, not improves, maximal insulin-stimulated glucose transport in skeletal muscle of different fibers. *Am J Physiol Regul Integr Comp Physiol* 299: R470–R479, 2010. First published June 10, 2010; doi:10.1152/ajpregu.00431.2009.—Agonists targeting the nuclear receptor peroxisome proliferator-activated receptors (PPAR)-δ may be potential therapeutic agents for insulin-resistant related conditions, as they may be able to stimulate fatty acid (FA) oxidation and attenuate the accumulation of harmful lipid species in skeletal muscle. Several reports have demonstrated that PPAR-δ agonists improve whole body insulin sensitivity. However, whether these agonists exert their direct effects on glucose and FA metabolism in skeletal muscle, and specifically with different fiber types, is unknown. This study was undertaken to determine the effects of oral treatment with the PPAR-δ agonist, GW 501516, in conjunction with the administration of a high-saturated-fat diet on insulin-stimulated glucose transport in isolated oxidative (soleus) and glycolytic (epitrochlearis) rodent skeletal muscle in vitro. High-fat feeding significantly decreased maximal insulin-stimulated glucose transport in soleus, but not epitrochlearis muscle, and was associated with increased skeletal muscle diacylglycerol and ceramide content. Unexpectedly, treatment with the PPAR-δ agonist significantly reduced insulin-stimulated glucose transport in both soleus and epitrochlearis muscles, regardless of dietary fat content. The reduction in insulin-stimulated glucose transport induced by the agonist was associated with large increases in total muscle fatty acid translocase (FAT)/CD36protein content, but not diacylglycerol or ceramide contents. Agonist treatment did not alter the protein content of PPAR-δ, GLUT4, or insulin-signaling proteins (IRS-1, p85 PI3-K, Akt). Agonist treatment led to a small, but significant increase, in the oxidative capacity of glycolytic but not oxidative muscle. We propose that chronic treatment with the PPAR-δ agonist GW 501516 may induce or worsen insulin resistance in rodent skeletal muscle by increasing the capacity for FA transport across the sarcolemma without a sufficient compensatory increase in FA oxidation. However, an accumulation of diacylglycerol and ceramide, while associated with diet-induced insulin resistance, does not appear to be responsible for the agonist-induced reduction in insulin-stimulated glucose transport.

fatty acid transport; oxidative capacity; diacylglycerol; ceramide; insulin resistance; high-fat diet

PEROXISOME PROLIFERATOR-ACTIVATED RECEPTORS (PPARs) are a subclass of nuclear receptors found in most tissues and have been implicated as important regulators of glucose and lipid metabolism. Natural ligands for the PPARs include fatty acids (FAs) (17), eicosanoids (52), prostaglandins (19), and leukotrienes (13). PPAR-α was the first of three isoforms to be identified and plays a critical role in FA uptake and metabolism by modulating the expression of genes for FA transport protein (FATP1), acyl-CoA oxidase, and long chain acyl-CoA synthetase (1, 35, 47). PPAR-α is expressed predominantly in the liver and to a lesser extent in skeletal muscle, kidney, and heart (4). Synthetic agonists for PPAR-α include the fibrate family of drugs, such as clofibrate and fenofibrate (33). PPAR-γ is highly expressed in adipose tissue and regulates differentiation, maturation, and lipid storage (24) and to a much lesser extent in skeletal muscle, heart, liver, and intestine (1). PPAR-γ is a target of the anti-diabetic drugs, thiazolidinediones (39). PPAR-δ is ubiquitously expressed and is a key regulator of lipid metabolism in skeletal muscle (2, 43, 50) and induces the transcription of genes for FATP1, long-chain acyl-CoA dehydrogenase, carnitine palmitoyl transferase I, and long chain acyl-CoA synthetase (43). Overexpressing PPAR-δ in rodent skeletal muscle leads to a significant increase in oxidative capacity in muscles characterized predominantly by glycolytic fibers (29, 50). Interestingly, the effect of PPAR-δ agonists in oxidative muscles is much less well established, with little demonstration of improved oxidative capacity (10). Several selective agonists have been developed for this isoform, including GW 501516 and GW 0742 (32, 42). Thus, PPARs are important regulators of lipid metabolism in many peripheral tissues and are a target of several pharmacological agents aimed at improving glucose tolerance.

Decreased insulin-stimulated glucose uptake into skeletal muscle is a hallmark characteristic of type 2 diabetes and is associated with dysregulated FA metabolism. Several studies have examined the consequence of chronic PPAR-δ agonist administration on insulin sensitivity. Results of both animal (28, 43, 50) and human (34) studies using PPAR-δ agonists have demonstrated improved whole-body glucose tolerance. However, studies examining the direct effect of PPAR-δ agonists on skeletal muscle have been generally limited to acute periods of exposure to isolated muscles/cell lines, with inconsistent results. In terms of glucose metabolism, findings have ranged from stimulation of glucose uptake (26, 27), no effect on glucose uptake or glycogen synthesis (12, 14, 44), and decreases in glucose uptake (6). The acute effects of PPAR-δ (GW 50156) stimulation on AMPK activation have also been equivocal (26, 27, 44). The reason for these discrepancies is not clear but may be related to the use of different models (cells vs. mature skeletal muscle), muscle fiber type (oxidative vs. glycolytic), and the duration of exposure to the agonist.

Address for reprint requests and other correspondence: David J Dyck, Dept. of Human Health and Nutritional Sciences, Univ. of Guelph, Ontario, Canada, N1G 2W1 (e-mail: ddyck@uoguelph.ca).

Table 1. *PPAR-δ agonist increases oxidative capacity markers in glycolytic, but not oxidative muscle in control and high-fat fed rats*

	Control Diet	Control Diet + Agonist	High-Fat Diet	High-Fat Diet + Agonist
Citrate Synthase				
Soleus	43.2 ± 2.4	39.5 ± 2.4	38.2 ± 2.1	46.4 ± 1.4
Red gastrocnemius	61.9 ± 3.6	64.2 ± 4.4	61.5 ± 3.3	61.4 ± 4.0
White gastrocnemius	21.0 ± 1.2	30.0 ± 1.4*	27.8 ± 2.2*	29.2 ± 1.4*
Beta-hydroxy acyl dehydrogenase				
Soleus	15.6 ± 0.9	15.1 ± 1.0	17.4 ± 0.9	16.7 ± 0.9
Red gastrocnemius	13.0 ± 0.9	15.2 ± 0.8	13.3 ± 1.0	16.4 ± 1.1
White gastrocnemius	6.0 ± 1.2	9.0 ± 0.6*	9.4 ± 1.8*	10.0 ± 1.4*

Values are expressed as means ± SE. Enzymes activities are in μmol/g wet wt/min. *Significantly different from control group ($P < 0.05$); $n = 6–8$ animals per group.

Given the importance of muscle lipid metabolism as a modulator of insulin response, it is surprising that there has apparently been no examination of the effects of chronic PPAR-δ agonist administration on muscle FA transporters and the content of reactive lipid species such as diacylglycerol (DAG) and ceramide. Therefore, the purpose of the present study was to determine the effects of chronic treatment with the PPAR-δ agonist, GW 501516, on insulin-stimulated glucose transport in isolated skeletal muscle under in vitro conditions, from normal and high-fat fed rats. Specifically, we hypothesized that chronic agonist treatment could reverse the insulin resistance induced by a high-fat diet (30, 37) and that this rescue of insulin response would be associated with improvements in muscle oxidative capacity and changes in muscle FA metabolism that would be expected to improve insulin response, i.e., reduced expression of FA transporter proteins [fatty acid translocase (FAT)/CD36 and fatty acid binding protein (FABPpm)] and reduced content of reactive lipid species (DAG, ceramide). We also wanted to determine whether there were any fiber-type differences in response to treatment with the high-fat diet and agonist, as there have been reports of fiber-type differences in response to high-fat-induced insulin resistance in skeletal muscle (38, 53).

METHODS

Animals and diets. Female Sprague-Dawley rats, weighing 130 to 150 g, were obtained from Charles River Inc (Quebec, Canada) and used for all experiments. Upon arrival, rats were assigned to individual cages in a controlled environment with a reversed 12-h light-dark cycle, where they were allowed to acclimatize and provided ad libitum access to standard rat chow and water. After acclimating for 3 days, rats were randomly assigned to one of four experimental groups: control diet (12% kcal from fat), high-fat diet (60% kcal from lard), control diet treated with GW 501516, and high-fat diet treated with GW 501516. Diets were obtained from Research Diets (New Brunswick, NJ). The University of Guelph Animal Care Committee approved all experimental protocols involving animal use.

Rats in the experimental groups were fed their respective diets for a total of 4 wk. For agonist-treated groups, GW 501516 was dissolved in DMSO at a concentration of 5 mg/ml and supplemented in the diet at a dose of 4 mg/kg body mass for the last 2 wk of the feeding trial. DMSO (without the agonist) was added to the nonagonist diets. High-fat fed rats were pair-fed with rats on the control diet to minimize differences in body mass gain. Prior to all experimental procedures, rats were fasted overnight and anesthetized with an intraperitoneal injection of pentobarbital sodium (6 mg/kg body mass).

Skeletal Muscle Measurements

Oxidative enzyme capacity. We determined the effect of 2 wk of agonist feeding, with or without the high-fat diet, on citrate synthase (CS) and 3-hydroxyacyl-CoA dehydrogenase (β-HAD) activity in muscles of oxidative (soleus), glycolytic (white gastrocnemius), and mixed (red gastrocnemius) fiber composition. Approximately 10 mg of muscle was homogenized in a 0.1 M potassium phosphate buffer solution (pH 7). Maximal CS and β-HAD activity were assayed spectrophotometrically as previously described (40).

Glucose transport. Epitrochlearis (glycolytic) and soleus (oxidative) muscles were used for the determination of basal and insulin-stimulated glucose transport. The soleus was carefully blunt-dissected into longitudinal strips from tendon to tendon using a 27-gauge needle; epitrochlearis was removed in its entirety. After excision from the animal, epitrochlearis and soleus muscles were incubated for 30 min at 30°C in 2 ml of pregassed (95% O_2-5% C O_2) Krebs-Henseleit buffer (KHB) containing 0.1% BSA, 8 mM glucose and 32 mM mannitol. Muscles were then washed twice for 10 min in a glucose-free KHB buffer containing 4 mM pyruvate and 36 mM mannitol, and finally incubated for 20 min (insulin-stimulated) or 40 min (basal) in KHB containing 4 mM pyruvate, 8 mM 3-O-[^{3}H]methyl-D-glucose (800 Ci/mmol), and 28 mM [^{14}C]mannitol (60 Ci/mmol). After all incubations, muscles were trimmed of tendons, blotted, and weighed. Muscle samples were then digested in 1 ml of 1 M NaOH for 10 min

Table 2. *Body mass and fasting blood measurements*

Parameter	C	C+GW	HF	HF + GW
Body mass pretrial, g	134 ± 4	142 ± 4	140 ± 4	138 ± 4
Body mass posttrial, g	222 ± 6	212 ± 7	231 ± 6	221 ± 8
Plasma insulin, ng/ml	4.4 ± 0.1	4.6 ± 0.1	4.7 ± 0.1	4.7 ± 0.1
Blood glucose, mmol/l	6.5 ± 0.3	8.1 ± 0.5*	8.2 ± 0.6†	9.0 ± 0.6*,†
Plasma FFA, mmol/l	0.38 ± 0.03	0.44 ± 0.04	0.40 ± 0.04	0.42 ± 0.04

Values are expressed as means ± SE. C, control; HF, high-fat; C + GW, control treated with agonist; HF + GW; high-fat treated with agonist; FFA, free fatty acids. *Significantly different from nonagonist groups ($P < 0.05$). †Significantly different from control groups ($P < 0.05$); $n = 8$ to 10 animals per group.

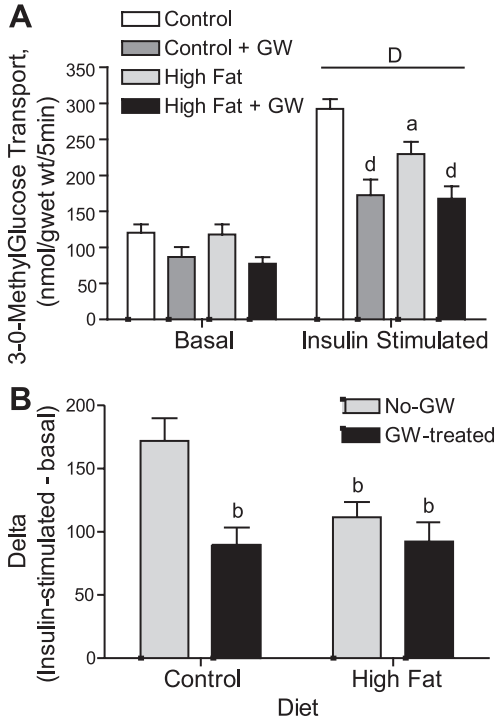

Fig. 1. *A*: basal and insulin-stimulated glucose transport. *B*: calculated increase in glucose transport rate from basal in response to insulin stimulation in soleus muscle from control, control + GW 501516 (GW), high fat, and high fat + GW treatment groups. Values are expressed as mean ± SE. [a]Significantly different from control group ($P < 0.05$). [b]Significantly different from control group ($P < 0.01$).[d]Significantly different than control group ($P < 0.0001$). [D]Significantly different from basal conditions ($P < 0.0001$); $n = 8$ to 10 animals per group.

at 95°C. Two-hundred microliters of the digested muscle was sampled in duplicate for liquid scintillation counting.

Insulin-signaling proteins. Epitrochlearis and soleus muscles were incubated in KHB similar to that used for the determination of glucose transport (without radioisotopes), for 10 min in the presence or absence of insulin (10 mU/ml) for the determination of the transient phosphorylation of key insulin signaling proteins (Akt, AS160). After incubation, the strips were immediately frozen and stored in liquid N_2 until subsequent Western blot analyses.

Western blot analyses. Muscles (30–50 mg) were homogenized on ice in a buffer suitable for the extraction of cytosolic and membrane-bound proteins, containing 50 mM Tris (pH 7.5), 1 mM EDTA, 1 mM EGTA, 50 mM NaF, 5 mM sodium pyrophosphate, 10% (vol/vol) glycerol, and 1% (vol/vol) Triton X-100 The homogenized samples were centrifuged at 20,000 *g* at 4°C for 20 min for collection of the supernatant. Fifty micrograms of the muscle lysate protein was solubilized in 4× Laemmeli's buffer and boiled at 95°C. Samples were then resolved by SDS-PAGE and wet transferred to polyvinylidene difluoride (PVDF) membranes (1 h at 100 V). Membranes were then blocked for ~1 to 2 h and incubated overnight at 4°C with antibodies specific for the following proteins: PPAR-δ (Abcam, Cambridge, MA), total Akt (Millipore, Temecula, California, USA), total IRS-1 (Millipore, Temecula, CA), PI3-K p85 (Millipore), FAT/CD 36, $FABP_{pm}$, (kindly provided by A. Bonen) and GLUT4 (Millipore), Ser^{473} and Thr^{308} phosphorylated Akt, (Santa Cruz Biotechnology, Santa Cruz, CA), and Thr^{642} phosphorylated Akt substrate 160 (pAS160; Medicorp, Montreal, QC, Canada). After incubation with the primary antibody, membranes were washed, incubated with the appropriate secondary antibody, and washed once more. The immune complexes were detected using enhanced chemiluminnescence and each band was quantified using densiometry (ChemiGenius 2 Bioimaging system; Syngene, Cambridge, UK). Confirmation of equal loading was done using nonspecific protein staining with Ponceau-S. Where appropriate, quantification of α-tubulin (Abcam, Cambridge, MA) was also used to verify equal loading.

Muscle lipid content. The red and white sections of the gastrocnemius were used for the purpose of this measurement. One-hundred milligrams of muscle was freeze-dried, powdered, and cleared of any visible connective tissue. Lipids were extracted in a chloroform-methanol solution, as described by Folch et al. (18). Diacylglycerol and ceramides were extracted and quantified by thin-layer chromatography/mass spectroscopy, as has been previously described (5). Total DAG and ceramide content were expressed in nanomoles per gram of dry mass muscle tissue.

Blood collection and analyses. At the completion of the 4-wk of feeding, fasting blood samples were collected following all surgical procedures by cardiac puncture. Blood samples were immediately transferred to a sodium-heparinized tube and centrifuged at 20,000 *g* for 20 min at 4°C. Plasma was removed and stored at −80°C until analyzed. Whole blood glucose was assessed with a glucometer (Bayer Elite XL, Toronto, Canada). Plasma insulin concentrations were assessed by radioimmunoassay (Millipore), and free fatty acids were determined using an enzymatic colorimetric method (NEFA C test kit, Wako, Richmond, Virginia).

Calculation and statistics. Glucose transport in soleus and epitrochlearis muscles was calculated as the intracellular accumulation of 3-O-[^{3}H] methyl-D-glucose after correcting for the interstitial volume with [^{14}C] mannitol. The data are presented as means ± SE. Differences in basal and insulin-stimulated glucose transport between groups were analyzed using a repeated-measures ANOVA. Additionally, insulin-stimulated glucose transport was analyzed using a two-

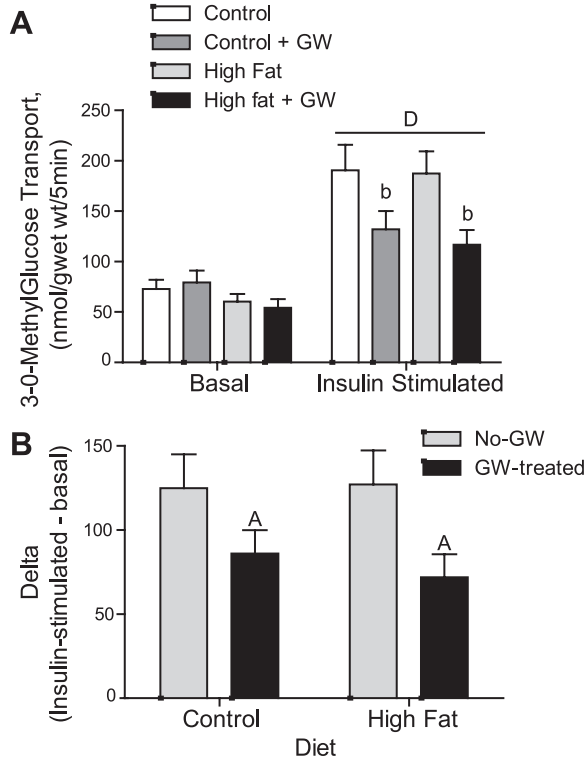

Fig. 2. *A*: Basal and insulin-stimulated glucose transport. *B*: calculated increase in glucose transport rate from basal in response to insulin stimulation in epitrochlearis muscle from control, control + GW, high fat, and high fat + GW treatment groups. Values are expressed as means ± SE. [A]Significantly different from non-GW-treated groups ($P < 0.05$); [b]Significantly different from non-GW-treated groups ($P < 0.01$); [D]Significantly different basal conditions ($P < 0.0001$); $n = 8$ to 10 animals per group.

way ANOVA to compare the soleus and epitrochlearis muscles. All other experimental measurements were analyzed using a two-way ANOVA, with a Student Newman-Keuls post hoc test being used to detect significant differences revealed by the two-way ANOVA. Statistical significance was accepted as $P < 0.05$.

RESULTS

Oxidative enzyme capacity. Four weeks of high-fat diet increased markers of maximal oxidative capacity (citrate synthase, CS) and FA oxidation (β-HAD) in the white gastrocnemius (glycolytic), but not the more oxidative soleus and red gastrocnemius muscles (Table 1). Inclusion of the agonist for the final 2 wk resulted in a small, but significant, increase in CS and β-HAD in white gastrocnemius, but not soleus or red gastrocnemius muscles.

Body mass and blood measurements. There were no significant differences in pretrial or posttrial body mass among the groups (Table 2), as anticipated with the pair-feeding design. There was no significant effect of diet or agonist treatment on fasting plasma insulin concentrations. However, both high-fat diet and agonist treatment led to a significant increase in fasting whole blood glucose concentrations ($P < 0.05$; Table 2). There were no significant differences in fasting plasma free fatty acid concentrations in response to diet (Table 2), similar to other studies in our laboratory (data unpublished). Agonist treatment also had no effect on plasma free fatty acid concentrations.

Basal and insulin-stimulated glucose transport. Basal rates of glucose transport in soleus (Fig. 1*A*) and epitrochlearis (Fig. 2*A*) muscles were similar in all conditions. Insulin stimulation significantly increased glucose transport in both muscles ($P < 0.0001$). Compared with the control group, there was a significant attenuation of insulin-stimulated glucose transport in soleus muscle in agonist (−33%, $P < 0.0001$), high-fat (−21%, $P < 0.05$) and high-fat + agonist (−43%, $P < 0.0001$) groups. In contrast, in the epitrochlearis, there was no significant difference in insulin-stimulated glucose transport between the control and high-fat group. However, there was a significant decrease in insulin-stimulated glucose transport in the agonist (−31%, $P < 0.01$) and high-fat + agonist (−39%, $P < 0.01$) groups compared with control. In neither muscle

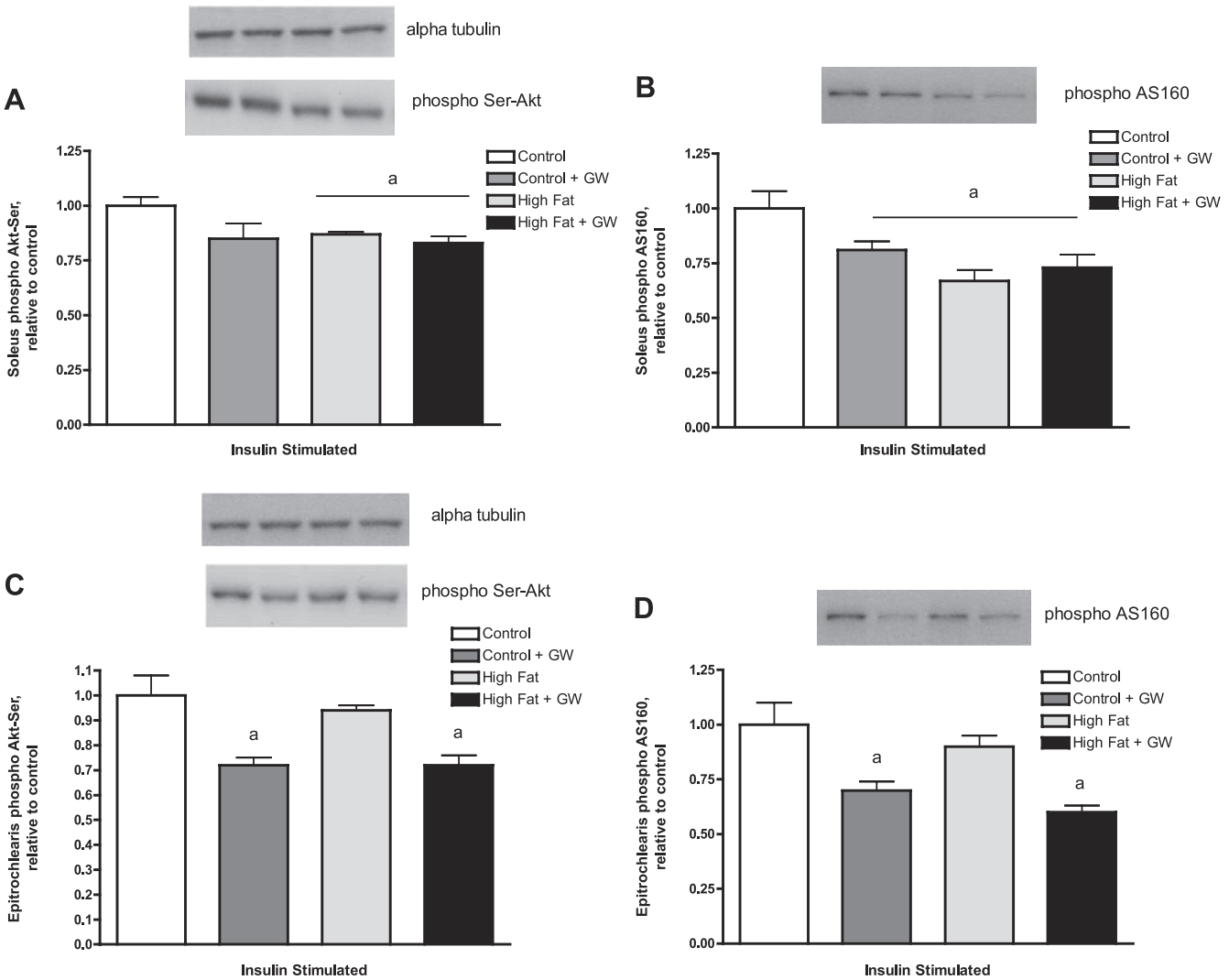

Fig. 3. Representative blots and quantification of Akt (*A* and *C*) and AS160 (*B* and *D*) phosphorylation during an acute insulin challenge in soleus (*A* and *B*) and epitrochlearis (*C* and *D*) muscles from control, high-fat, control + GW, and high-fat + GW groups. Values are expressed as means ± SE, expressed as relative to control values. [a]Significantly different from control group ($P < 0.05$); $n = 8$ animals per group.

was there any evidence of an additive effect of the high-fat diet and agonist treatment.

The difference (delta) between basal and maximal insulin-stimulated glucose transport rates in soleus and epitrochlearis muscles were calculated for all groups. In the soleus (Fig. 1*B*), the delta for the agonist, high-fat, and high-fat + agonist groups were significantly lower ($P < 0.01$) than that of the control group (−47%, −34%, −46%, respectively). In the epitrochlearis (Fig. 2*B*), delta values were significantly reduced in agonist (−31%, $P < 0.05$) and high fat + agonist (−42%, $P < 0.05$) groups; there was no significant difference between the control and high-fat group.

Western Blot Analyses

Skeletal muscle insulin signaling protein phosphorylation and total content. The transient phosphorylation of key insulin signaling proteins, Akt and AS160, was significantly blunted during acute insulin stimulation in both soleus and epitrochlearis muscles following agonist treatment, regardless of the diet (Fig. 3). High-fat diet alone caused impaired insulin signaling in the oxidative soleus muscle only. These findings essentially mirror those of the insulin-stimulated glucose transport. There were no significant differences among the groups, due to either diet or agonist treatment, with respect to the total protein contents of IRS-1, PI3-K p85, Akt, or AS160 (data not shown) in either oxidative (soleus) or glycolytic (epitrochlearis, WG).

Skeletal muscle PPAR-δ protein expression. Treatment with the high-fat diet, but not agonist, significantly increased PPAR-δ protein content in soleus ($P < 0.05$; Fig. 4*A*). There was no effect of either high-fat diet or agonist treatment on the PPAR-δ protein in WG (Fig. 4*B*).

Skeletal muscle FABPpm and FAT/CD36 protein expression. There was no effect of high-fat diet or agonist treatment on total FABPpm protein content in soleus and WG (Fig. 5). However, both high-fat diet and agonist led to a significant increase in FAT/CD36 protein content in soleus and WG ($P < 0.01$). Treatment with the agonist was quite profound, causing a near doubling of total FAT/CD36 content in both muscles compared with control, similar to that observed with the high-fat diet. The effects of diet and agonist treatments were not additive.

Skeletal muscle GLUT4 protein expression. There was no effect of diet or agonist on whole muscle GLUT4 protein content in soleus or WG (Fig. 6).

Skeletal muscle ceramide and DAG content. There was a significant increase ($P < 0.05$) in ceramide content in the red gastrocnemius (RG) muscle as a result of the high-fat diet (Fig. 7). Agonist treatment alone had no effect on ceramide content in RG. Muscle ceramide content in the WG was unaffected by diet and agonist. Diacylglycerol content was significantly increased in the RG of agonist ($P < 0.01$), high-fat ($P < 0.01$), and high-fat + agonist ($P < 0.05$) groups. Muscle DAG content was unaffected in the WG by diet and agonist.

DISCUSSION

Our major finding was that oral administration of the PPAR-δ agonist, GW 501516, significantly reduced maximal insulin-stimulated glucose transport and insulin signaling in skeletal muscle. This reduction was independent of diet and occurred in both oxidative and glycolytic muscles.

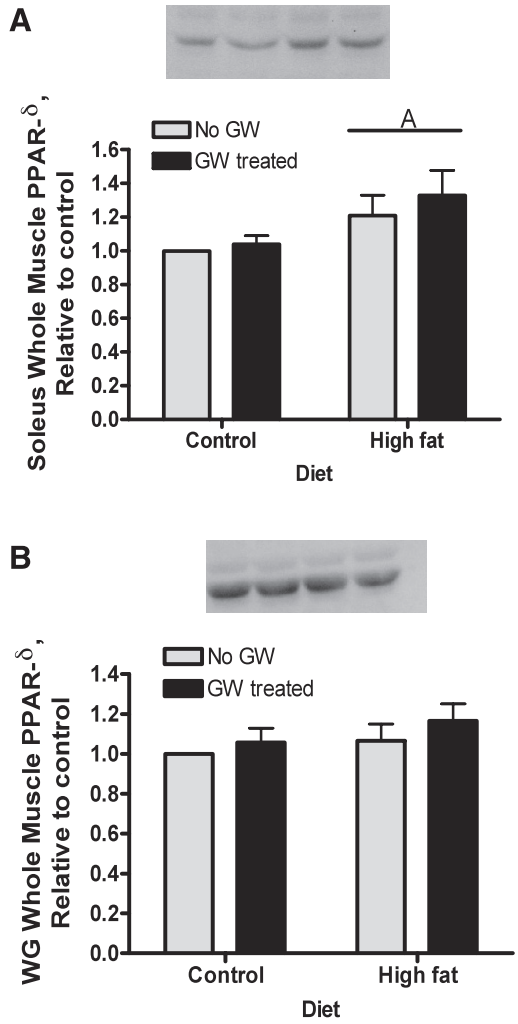

Fig. 4. Representative blot and quantification of peroxisome proliferator-activated receptors-δ (PPAR-δ) protein expression in soleus (*A*) and white gastrocnemius (*B*; WG) muscle homogenates from control, high-fat, control + GW, and high-fat + GW groups. Values are expressed as means ± SE. [A]Significantly different from control diet ($P < 0.05$); $n = 8$ to 10 animals per group.

The impaired glucose transport was not due to changes in the muscle protein content of GLUT4 or specific insulin signaling molecules (p85 PI3-K, IRS-1, Akt). Agonist and diet treatment independently increased the total protein content of the FA transporter FAT/CD36 in both oxidative and glycolytic muscles. This increase in transporter coincided with elevated DAG and ceramide content in high-fat fed rats; surprisingly, these lipids were generally not increased (or further increased relative to the high-fat diet) in agonist-treated rodents. Taken together, the results from our study suggest that chronic treatment with the PPAR-δ agonist, GW 501516, may induce or worsen insulin response in rodent skeletal muscle. This appears to be related to a large increase in total FAT/CD36 content; however, the lack of increase in muscle DAG or ceramide content suggests that a potential accumulation of other lipid species could be a cause of impaired insulin response. Alternatively, we cannot rule out a possible direct and unexpected impairment of either the GLUT4 transporters or insulin signaling proteins caused by the agonist.

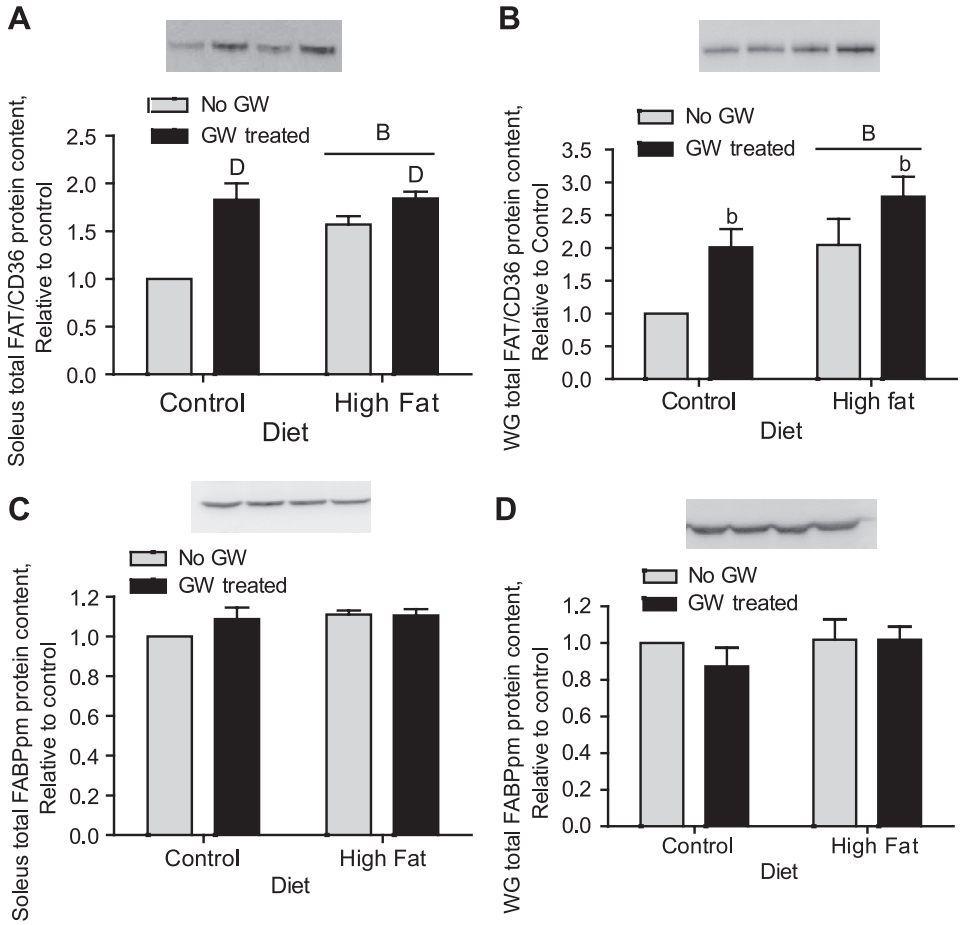

Fig. 5. Representative blots and quantification of fatty acid translocase (FAT)/CD36 (*A* and *B*) and fatty acid binding protein (FABPpm) (*C* and *D*) protein expression in soleus and white WG muscle homogenates from control, high-fat, control + GW, and high-fat + GW groups. Values are expressed as means ± SE, expressed as relative to control values; $n = 8$ to 10 animals per group. [B]Significantly different from control diet: [D]Significantly different from basal conditions ($P < 0.0001$).

Oxidative Capacity and FA Metabolism

Oxidative capacity. Several studies have implicated PPAR-δ as a regulator of oxidative capacity in skeletal muscle (29, 50). However, the studies reporting this finding have generally used muscles composed primarily of glycolytic fibers (50); information pertaining to oxidative muscle is limited. On the basis of our results, it would appear that glycolytic fibers respond more robustly than oxidative fibers to treatment with GW 501516 in terms of increasing their capacity for oxidative metabolism. It is possible that higher doses of agonist may have elicited significant increases in oxidative capacity in the soleus muscle. We chose to use a low dose of agonist (4 mg/kg body mass), as higher doses have been reported to cause hepatomegaly (43). Interestingly, there was a divorcing of changes in oxidative markers with changes in PPAR-δ protein content. PPAR-δ protein content in oxidative muscle increased in response to a high-fat diet (albeit modestly, ~20 to 30%), but this did not translate to a significant increase in CS or β-HAD; conversely, oxidative markers increased in glycolytic muscle in response to the agonist, in spite of no change in PPAR-δ content. Thus, it seems likely that changes in PPAR-δ protein are not necessarily reflective of its activity.

FA metabolism. FA transport, facilitated by FAT/CD36 and FABPpm, is a key step in the regulation of FA metabolism in skeletal muscle. In sedentary rodents and humans, there is an increase in sarcolemmal FA transporter content which is associated with intramuscular lipid accumulation and insulin resistance (3, 8). Several studies have examined the effects of PPAR-δ agonists on the mRNA expression (but not protein content) of FAT/CD36 in muscle cell lines (15, 49) and macrophages (48), generating mixed results. In the present study, agonist administration significantly increased muscle FAT/CD36 protein content in both oxidative and glycolytic muscles, similar to that observed with the high-fat diet. Our results provide a possible mechanism linking agonist treatment with the observed impairment of insulin-stimulated glucose transport in the present study. Unfortunately, we were unable to measure sarcolemmal transporter protein content in the present study due to tissue limitations. It is likely, however, that the increases in total FAT/CD36 observed in the present study coincided with a parallel increase in the sarcolemmal content, as several reports have indicated that increases in whole muscle FAT/CD36 are concurrent with increases in its sarcolemmal content (7, 22, 37). We have no explanation as to why FABPpm content was unaffected by agonist treatment. However, recent findings of Nickerson et al. (31) clearly demonstrate a greater transport efficiency of FAT/CD36 relative to FABPpm. Arguably, then, we have demonstrated significant increases in the more important FA transporter. While it is tempting to link the agonist-induced increase in FAT/CD36 with the presence of insulin resistance, there are two obvious caveats. First, the increase in FA transporters did not necessarily lead to an increased intramuscular content of reactive lipid species (discussed below). Second, this potential

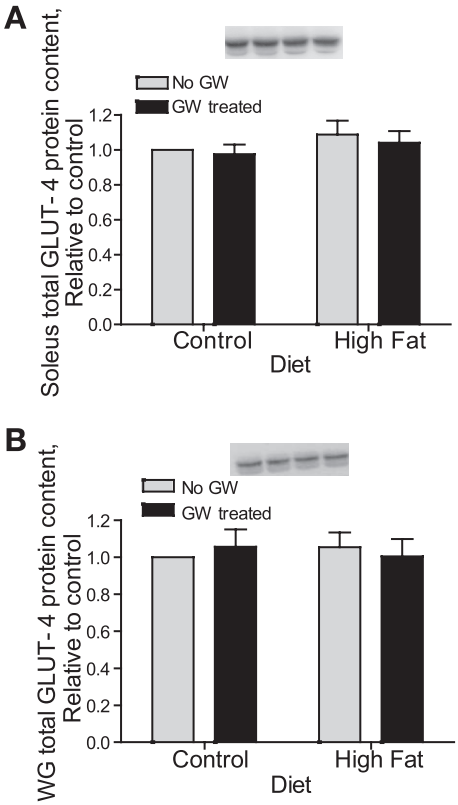

Fig. 6. Representative blots and quantification of GLUT4 protein expression in soleus (*A*) and WG (*B*) muscle homogenates from control, high-fat, control + GW, and high-fat + GW groups. Values are expressed as means ± SE, expressed as relative to control values; $n = 8–10$ animals per group.

association can be divorced in the glycolytic muscle, which shows a twofold increase in FAT/CD36 in the high fat condition, but no impairment in insulin signaling or glucose transport.

Skeletal muscle lipid content. The accumulation of reactive lipid species, including DAG and ceramides, may be a key factor in the high-fat-induced impairment of insulin-stimulated glucose transport in skeletal muscle (41, 45). In the present study, we show that the high-fat diet significantly increased DAG and ceramide content in red, but not white gastrocnemius, muscles. These relative changes in DAG and ceramide content were similar to the direction of change in maximal insulin response in soleus (impaired response) and epitrochlearis (no impairment) muscles. However, in contrast to the effect of the high-fat diet, agonist treatment generally did not alter muscle lipid content (with the exception of DAG in the RG), whether administered alone or in conjunction with the high-fat diet. Clearly, changes in DAG/ceramide cannot be implicated as the main cause of agonist-induced impairment of insulin-stimulated glucose transport. This is particularly puzzling given the observed increase in total FAT/CD36 transporter content, which would presumably have led to increases in these lipids. However, it is possible that other reactive lipid species were increased, which we did not measure. Alternatively, it is possible that the PPAR agonist had other nonselective effects, including a direct effect on either the GLUT4 protein or the ability of insulin-signaling proteins to respond to an insulin challenge, independent of any effect on the FA transporters or intramuscular lipid content. There is also recent evidence that the administration of a PPAR-δ agonist to rats increases pyruvate dehydrogenase kinase activity in skeletal muscle, resulting in significant impairment of carbohydrate oxidation both at rest and during contraction (11). While this may not directly explain our impairment of a nonmetabolizable glucose analog under an insulin-stimulated condition in isolated muscle, it does support the notion that these agonists may have unexpected and detrimental effects on glucose metabolism.

Glucose Homeostasis and Skeletal Muscle Insulin-Stimulated Glucose Transport

Blood glucose. There was a significant increase in fasting blood glucose concentrations due to both agonist treatment and high-fat diet. Our finding that agonist treatment significantly increased fasting blood glucose was unexpected and in contrast to previous studies (28, 34, 43). It is possible that variations in experimental design such as dosage and duration of GW 501516 administration, as well as species selection (rats vs. mice), accounted for these differences. Nevertheless, the agonist-induced increase in blood glucose is certainly reflective of our observed impairment in insulin-stimulated glucose transport in skeletal muscle.

Glucose transport in skeletal muscle. A number of studies have shown that chronic treatment with a high-fat diet induces insulin resistance in rodent skeletal muscle, which may be due, in part, to increasing the content of reactive lipid species (37, 53). Consistent with our own previous findings (30, 37), we showed that the administration of a high-fat diet for 4 wk reduced maximal insulin-stimulated glucose transport in soleus muscle. Unfortunately, there was insufficient soleus and epitrochlearis tissue to permit lipid analyses in these muscles; however, we observed a significant increase in total DAG and ceramide contents in the oxidative red gastrocnemius. In contrast, high-fat treatment did not reduce insulin response in the epitrochlearis muscle. This finding may not be surprising as oxidative muscles are potentially more susceptible to developing FA-mediated insulin resistance (51, 53). Furthermore, plasma membrane FAT/CD36 protein content is increased in oxidative, but not glycolytic, muscles of insulin-resistant Zucker Diabetic Fatty rats (7), and excess lipid accumulation occurs in oxidative but not glycolytic muscles of obese and type 2 diabetic humans (20). In the present study, the administration of a high-fat diet increased total expression of FAT/CD36 in both fiber types; however, accumulation of DAG and ceramide and a decrease in insulin response was only observed in oxidative muscles. It is possible that the increase in total transporter content did not reflect similar changes in the plasma membrane. In our view, it is more likely that there was an adaptive increase in FA oxidation in glycolytic muscle in response to the high-fat diet that was sufficient to compensate for an increase in FA uptake, although this was not directly assessed.

It has been proposed that treatment with a PPAR-δ agonist may be able to attenuate the accumulations of harmful lipid species in skeletal muscle on the basis of its ability to induce significant increases in FA oxidation (43, 49). As such, we predicted that agonist treatment would rescue the decrease in insulin-stimulated glucose transport in skeletal muscle induced

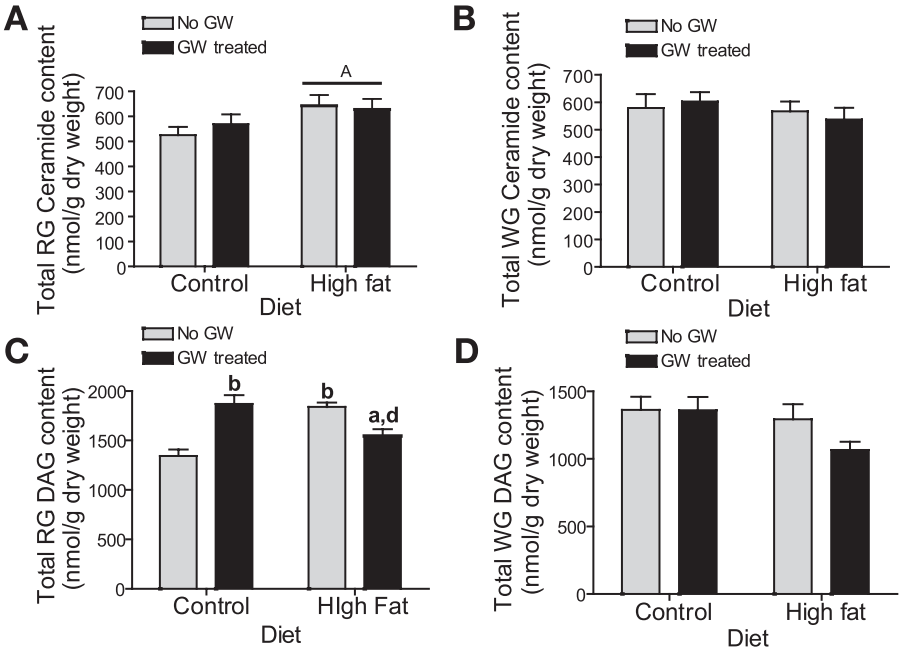

Fig. 7. Total ceramide (*A* and *B*) and diacylglycerol (*C* and *D*; DAG) contents in red (*A* and *C*; RG) and white (*B* and *D*; WG) gastrocnemius muscles from control, high fat, control + GW, and high fat + GW groups. Values are expressed as means ± SE. [A]Significantly different from control treated groups ($P < 0.05$); $n = 7$–9 animals per group. [a]Significantly different from control group ($P < 0.05$); $n = 8$ animals per group. [b]Significantly different from groups without GW treatment ($P < 0.01$). [d]Significantly different than control group ($P < 0.0001$).

by high-fat feeding. Unexpectedly, agonist administration led to a significant reduction in insulin-stimulated glucose transport in both soleus and epitrochlearis muscles, independent of diet treatment. The percentage decrease in insulin-stimulated glucose transport induced by the agonist was similar in both muscles, and mirrored by a blunted transient phosphorylation of Akt and AS160. This reduction in insulin-stimulated glucose transport in both muscle types was associated with significant increases in whole muscle FAT/CD36 content; however, DAG and ceramide content were not further elevated above that induced by the high-fat diet, even when the agonist was combined with the high-fat diet. It may be possible that other harmful lipid-derived species, such as long-chain acyl-CoAs or products of incomplete FA oxidation accumulated and were responsible for the impaired insulin response (9, 16).

Neither agonist treatment nor high-fat diet led to significant changes in the total protein content of measured insulin signaling proteins (IRS-1, PI3-K, Akt, or AS160) in either oxidative or glycolytic muscle. Previous studies have also shown no effect of high-fat feeding on the protein content of insulin signaling proteins in skeletal muscle (36, 53). To our knowledge, this is the first study to assess the effects of chronic treatment of a PPAR-δ agonist on the protein contents of these insulin-signaling proteins in skeletal muscle. The effect of PPAR-δ agonists on GLUT-4 mRNA expression in skeletal muscle is equivocal (23, 43). Similarly, the effect of a high-fat diet on muscle GLUT4 protein is controversial (21, 25, 36, 46). We found no influence of either high-fat diet or agonist on total GLUT4 protein in the present study.

Perspectives and Significance

This is the first study to examine the effects of chronic in vivo treatment with a PPAR-δ agonist (GW 501516) on insulin-stimulated glucose transport in skeletal muscle of different fiber types. Functional/metabolic assessments (glucose transport, insulin signaling) in this study were determined in oxidative (soleus) and glycolytic (epitrochlearis) muscles suitable for incubation under in vitro conditions. However, because of the small size of these muscles, it was necessary to use larger representative oxidative and glycolytic muscles to allow for the analyses of lipid content, fatty acid transporters, and various other regulatory enzymes and proteins. Nevertheless, it must be acknowledged that the utilization of these different muscles does represent a limitation to the current study. As we had anticipated, improved insulin response as a result of administration of the agonist, we assessed the metabolic outcome in rats fed a normal and high-fat, i.e., insulin resistance-inducing diet. Unexpectedly, GW 501516 administration reduced maximal insulin response in both soleus and epitrochlearis muscles. Although this effect was associated with a large increase in muscle FAT/CD36 content, the PPAR-δ agonist generally did not cause further accumulations of intramuscular DAG and ceramide beyond that induced by the high-fat diet. It is possible, however, that other harmful lipid-derived species may have accumulated. Although we believe that it is the activation of muscle PPAR-δ by the ingestion of saturated FA acids or the agonist that leads to the overexpression of FAT/CD36 and the impaired insulin response, we have not demonstrated this to be cause and effect or necessarily to be the only mechanism involved. Given the importance of skeletal muscle as a contributor to the regulation of blood glucose, it is essential to fully characterize how this tissue responds to treatment with a PPAR-δ agonist in vivo before these agents can be established as antidiabetic therapeutics.

ACKNOWLEDGMENTS

We gratefully acknowledge the technical assistance of Laelie Snook.

GRANTS

This research was funded by grants from the Natural Sciences and Engineering Research Council of Canada (to D. J. Dyck and A. Bonen), the Canadian Institutes of Health Research (to D. J. Dyck and A. Bonen) and Medical University of Bialystok (no. 3-18715L, AC).

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

REFERENCES

1. **Ahmed W, Ziouzenkova O, Brown J, Devchand P, Francis S, Kadakia M, Kanda T, Orasanu G, Sharlach M, Zandbergen F, Plutzky J.** PPARs and their metabolic modulation: new mechanisms for transcriptional regulation? *J Intern Med* 262: 184–198, 2007.
2. **Barish GD, Narkar VA, Evans RM.** PPAR delta: a dagger in the heart of the metabolic syndrome. *J Clin Invest* 116: 590–597, 2006.
3. **Bonen A, Parolin ML, Steinberg GR, Calles-Escandon J, Tandon NN, Glatz JF, Luiken JJ, Heigenhauser GJ, Dyck DJ.** Triacylglycerol accumulation in human obesity and type 2 diabetes is associated with increased rates of skeletal muscle fatty acid transport and increased sarcolemmal FAT/CD36. *FASEB J* 18: 1144–1146, 2004.
4. **Braissant O, Foufelle F, Scotto C, Dauca M, Wahli W.** Differential expression of peroxisome proliferator-activated receptors (PPARs): tissue distribution of PPAR-alpha, -beta, and -gamma in the adult rat. *Endocrinology* 137: 354–366, 1996.
5. **Bruce CR, Thrush AB, Mertz VA, Bezaire V, Chabowski A, Heigenhauser GJ, Dyck DJ.** Endurance training in obese humans improves glucose tolerance and mitochondrial fatty acid oxidation and alters muscle lipid content. *Am J Physiol Endocrinol Metab* 291: E99–E107, 2006.
6. **Brunmair B, Staniek K, Dorig J, Szocs Z, Stadlbauer K, Marian V, Gras F, Anderwald C, Nohl H, Waldhausl W, Furnsinn C.** Activation of PPAR-delta in isolated rat skeletal muscle switches fuel preference from glucose to fatty acids. *Diabetologia* 49: 2713–2722, 2006.
7. **Chabowski A, Chatham JC, Tandon NN, Calles-Escandon J, Glatz JF, Luiken JJ, Bonen A.** Fatty acid transport and FAT/CD36 are increased in red but not in white skeletal muscle of ZDF rats. *Am J Physiol Endocrinol Metab* 291: E675–E682, 2006.
8. **Chabowski A, Gorski J, Bonen A.** Regulation of fatty acid transport: from transcriptional to posttranscriptional effects. *Naunyn Schmiedebergs Arch Pharmacol* 373: 259–263, 2006.
9. **Chen MT, Kaufman LN, Spennetta T, Shrago E.** Effects of high fat-feeding to rats on the interrelationship of body weight, plasma insulin, and fatty acyl-coenzyme A esters in liver and skeletal muscle. *Metabolism* 41: 564–569, 1992.
10. **Constantin D, Constantin-Teodosiu D, Layfield R, Tsintzas K, Bennett AJ, Greenhaff PL.** PPARdelta agonism induces a change in fuel metabolism and activation of an atrophy programme, but does not impair mitochondrial function in rat skeletal muscle. *J Physiol* 583: 381–390, 2007.
11. **Constantin-Teodosiu D, Baker DJ, Constantin D, Greenhaff PL.** PPARdelta agonism inhibits skeletal muscle PDC activity, mitochondrial ATP production and force generation during prolonged contraction. *J Physiol* 587: 231–239, 2009.
12. **Debard C, Cozzone D, Ricard N, Vouillarmet J, Disse E, Husson B, Laville M, Vidal H.** Short-term activation of peroxysome proliferator-activated receptor beta/delta increases fatty acid oxidation but does not restore insulin action in muscle cells from type 2 diabetic patients. *J Mol Med* 84: 747–752, 2006.
13. **Delerive P, Furman C, Teissier E, Fruchart J, Duriez P, Staels B.** Oxidized phospholipids activate PPARalpha in a phospholipase A2-dependent manner. *FEBS Lett* 471: 34–38, 2000.
14. **Dimopoulos N, Watson M, Green C, Hundal HS.** The PPARdelta agonist, GW501516, promotes fatty acid oxidation but has no direct effect on glucose utilisation or insulin sensitivity in rat L6 skeletal muscle cells. *FEBS Lett* 581: 4743–4748, 2007.
15. **Dressel U, Allen TL, Pippal JB, Rohde PR, Lau P, Muscat GE.** The peroxisome proliferator-activated receptor beta/delta agonist, GW501516, regulates the expression of genes involved in lipid catabolism and energy uncoupling in skeletal muscle cells. *Mol Endocrinol* 17: 2477–2493, 2003.
16. **Ellis BA, Poynten A, Lowy AJ, Furler SM, Chisholm DJ, Kraegen EW, Cooney GJ.** Long-chain acyl-CoA esters as indicators of lipid metabolism and insulin sensitivity in rat and human muscle. *Am J Physiol Endocrinol Metab* 279: E554–E560, 2000.
17. **Ferre P.** The biology of peroxisome proliferator-activated receptors: relationship with lipid metabolism and insulin sensitivity. *Diabetes* 53 *Suppl* 1: S43–S50, 2004.
18. **Folch J, Lees M, Sloane Stanley GH.** A simple method for the isolation and purification of total lipides from animal tissues. *J Biol Chem* 226: 497–509, 1957.
19. **Forman BM, Chen J, Evans RM.** Hypolipidemic drugs, polyunsaturated fatty acids, and eicosanoids are ligands for peroxisome proliferator-activated receptors alpha and delta. *Proc Natl Acad Sci USA* 94: 4312–4317, 1997.
20. **He J, Kelley DE.** Muscle glycogen content in type 2 diabetes mellitus. *Am J Physiol Endocrinol Metab* 287: E1002–E1007, 2004.
21. **Herr HJ, Bernard JR, Reeder DW, Rivas DA, Limon JJ, Yaspelkis BB, 3rd.** Insulin-stimulated plasma membrane association and activation of Akt2, aPKC zeta and aPKC lambda in high fat fed rodent skeletal muscle. *J Physiol* 565: 627–636, 2005.
22. **Ibrahimi A, Bonen A, Blinn WD, Hajri T, Li X, Zhong K, Cameron R, Abumrad NA.** Muscle-specific overexpression of FAT/CD36 enhances fatty acid oxidation by contracting muscle, reduces plasma triglycerides and fatty acids, and increases plasma glucose and insulin. *J Biol Chem* 274: 26761–26766, 1999.
23. **Jucker BM, Yang D, Casey WM, Olzinski AR, Williams C, Lenhard SC, Legos JJ, Hawk CT, Sarkar SK, Newsholme SJ.** Selective PPARdelta agonist treatment increases skeletal muscle lipid metabolism without altering mitochondrial energy coupling: an in vivo magnetic resonance spectroscopy study. *Am J Physiol Endocrinol Metab* 293: E1256–E1264, 2007.
24. **Kliewer SA, Forman BM, Blumberg B, Ong ES, Borgmeyer U, Mangelsdorf DJ, Umesono K, Evans RM.** Differential expression and activation of a family of murine peroxisome proliferator-activated receptors. *Proc Natl Acad Sci USA* 91: 7355–7359, 1994.
25. **Koshinaka K, Oshida Y, Han YQ, Kubota M, Viana AY, Nagasaki M, Sato Y.** Insulin-nonspecific reduction in skeletal muscle glucose transport in high-fat-fed rats. *Metabolism* 53: 912–917, 2004.
26. **Kramer DK, Al-Khalili L, Guigas B, Leng Y, Garcia-Roves PM, Krook A.** Role of AMP kinase and PPARdelta in the regulation of lipid and glucose metabolism in human skeletal muscle. *J Biol Chem* 282: 19313–19320, 2007.
27. **Kramer DK, Al-Khalili L, Perrini S, Skogsberg J, Wretenberg P, Kannisto K, Wallberg-Henriksson H, Ehrenborg E, Zierath JR, Krook A.** Direct activation of glucose transport in primary human myotubes after activation of peroxisome proliferator-activated receptor delta. *Diabetes* 54: 1157–1163, 2005.
28. **Lee CH, Olson P, Hevener A, Mehl I, Chong LW, Olefsky JM, Gonzalez FJ, Ham J, Kang H, Peters JM, Evans RM.** PPARdelta regulates glucose metabolism and insulin sensitivity. *Proc Natl Acad Sci USA* 103: 3444–3449, 2006.
29. **Luquet S, Lopez-Soriano J, Holst D, Fredenrich A, Melki J, Rassoulzadegan M, Grimaldi PA.** Peroxisome proliferator-activated receptor delta controls muscle development and oxidative capability. *FASEB J* 17: 2299–2301, 2003.
30. **Mullen KL, Smith AC, Junkin KA, Dyck DJ.** Globular adiponectin resistance develops independently of impaired insulin-stimulated glucose transport in soleus muscle from high-fat-fed rats. *Am J Physiol Endocrinol Metab* 293: E83–E90, 2007.
31. **Nickerson JG, Alkhateeb H, Benton CR, Lally J, Nickerson J, Glatz JFC, Chabowski A, Luiken JJFP, Bonen A.** Greater transport efficiencies of the membrane fatty acid transporters and FAT/CD36 and FATP4 compared with FABPpm and FATP1 and diffential effects on fatty acid esterification and oxidation in rat skeletal muscle. *J Biol Chem* 284: 16522–16530, 2009.
32. **Oliver WR Jr., Shenk JL, Snaith MR, Russell CS, Plunket KD, Bodkin NL, Lewis MC, Winegar DA, Sznaidman ML, Lambert MH, Xu HE, Sternbach DD, Kliewer SA, Hansen BC, Willson TM.** A selective peroxisome proliferator-activated receptor delta agonist promotes reverse cholesterol transport. *Proc Natl Acad Sci USA* 98: 5306–5311, 2001.
33. **Peters JM, Hennuyer N, Staels B, Fruchart JC, Fievet C, Gonzalez FJ, Auwerx J.** Alterations in lipoprotein metabolism in peroxisome proliferator-activated receptor alpha-deficient mice. *J Biol Chem* 272: 27307–27312, 1997.
34. **Riserus U, Sprecher D, Johnson T, Olson E, Hirschberg S, Liu A, Fang Z, Hegde P, Richards D, Sarov-Blat L, Strum JC, Basu S, Cheeseman J, Fielding BA, Humphreys SM, Danoff T, Moore NR, Murgatroyd P, O'Rahilly S, Sutton P, Willson T, Hassall D, Frayn KN, Karpe F.** Activation of peroxisome proliferator-activated receptor (PPAR)delta promotes reversal of multiple metabolic abnormalities, reduces oxidative stress, and increases fatty acid oxidation in moderately obese men. *Diabetes* 57: 332–339, 2008.

35. **Schoonjans K, Watanabe M, Suzuki H, Mahfoudi A, Krey G, Wahli W, Grimaldi P, Staels B, Yamamoto T, Auwerx J.** Induction of the acyl-coenzyme A synthetase gene by fibrates and fatty acids is mediated by a peroxisome proliferator response element in the C promoter. *J Biol Chem* 270: 19269–19276, 1995.
36. **Singh MK, Krisan AD, Crain AM, Collins DE, Yaspelkis BB, 3rd.** High-fat diet and leptin treatment alter skeletal muscle insulin-stimulated phosphatidylinositol 3-kinase activity and glucose transport. *Metabolism* 52: 1196–1205, 2003.
37. **Smith AC, Mullen KL, Junkin KA, Nickerson J, Chabowski A, Bonen A, Dyck DJ.** Metformin and exercise reduce muscle FAT/CD36 and lipid accumulation and blunt the progression of high-fat diet-induced hyperglycemia. *Am J Physiol Endocrinol Metab* 293: E172–E181, 2007.
38. **Song XM, Kawano Y, Krook A, Ryder JW, Efendic S, Roth RA, Wallberg-Henriksson H, Zierath JR.** Muscle fiber type-specific defects in insulin signal transduction to glucose transport in diabetic GK rats. *Diabetes* 48: 664–670, 1999.
39. **Spiegelman BM.** PPAR-gamma: adipogenic regulator and thiazolidinedione receptor. *Diabetes* 47: 507–514, 1998.
40. **Srere PA.** Citrate synthase. *Methods Enzymol* 13: 3–11, 1969.
41. **Summers SA.** Ceramides in insulin resistance and lipotoxicity. *Prog Lipid Res* 45: 42–72, 2006.
42. **Sznaidman ML, Haffner CD, Maloney PR, Fivush A, Chao E, Goreham D, Sierra ML, LeGrumelec C, Xu HE, Montana VG, Lambert MH, Willson TM, Oliver WR Jr. Sternbach DD.** Novel selective small molecule agonists for peroxisome proliferator-activated receptor delta (PPARdelta)—synthesis and biological activity. *Bioorg Med Chem Lett* 13: 1517–1521, 2003.
43. **Tanaka T, Yamamoto J, Iwasaki S, Asaba H, Hamura H, Ikeda Y, Watanabe M, Magoori K, Ioka RX, Tachibana K, Watanabe Y, Uchiyama Y, Sumi K, Iguchi H, Ito S, Doi T, Hamakubo T, Naito M, Auwerx J, Yanagisawa M, Kodama T, Sakai J.** Activation of peroxisome proliferator-activated receptor delta induces fatty acid beta-oxidation in skeletal muscle and attenuates metabolic syndrome. *Proc Natl Acad Sci USA* 100: 15924–15929, 2003.
44. **Terada S, Wicke S, Holloszy JO, Han DH.** PPARdelta activator GW-501516 has no acute effect on glucose transport in skeletal muscle. *Am J Physiol Endocrinol Metab* 290: E607–E611, 2006.
45. **Timmers S, Schrauwen P, de Vogel J.** Muscular diacylglycerol metabolism and insulin resistance. *Physiol Behav* 94: 242–251, 2008.
46. **Tremblay F, Lavigne C, Jacques H, Marette A.** Defective insulin-induced GLUT4 translocation in skeletal muscle of high fat-fed rats is associated with alterations in both Akt/protein kinase B and atypical protein kinase C (zeta/lambda) activities. *Diabetes* 50: 1901–1910, 2001.
47. **Tugwood JD, Issemann I, Anderson RG, Bundell KR, McPheat WL, Green S.** The mouse peroxisome proliferator activated receptor recognizes a response element in the 5′ flanking sequence of the rat acyl CoA oxidase gene. *EMBO J* 11: 433–439, 1992.
48. **Vosper H, Patel L, Graham TL, Khoudoli GA, Hill A, Macphee CH, Pinto I, Smith SA, Suckling KE, Wolf CR, Palmer CN.** The peroxisome proliferator-activated receptor delta promotes lipid accumulation in human macrophages. *J Biol Chem* 276: 44258–44265, 2001.
49. **Wang YX, Lee CH, Tiep S, Yu RT, Ham J, Kang H, Evans RM.** Peroxisome-proliferator-activated receptor delta activates fat metabolism to prevent obesity. *Cell* 113: 159–170, 2003.
50. **Wang YX, Zhang CL, Yu RT, Cho HK, Nelson MC, Bayuga-Ocampo CR, Ham J, Kang H, Evans RM.** Regulation of muscle fiber type and running endurance by PPARdelta. *PLoS Biol* 2: e294, 2004.
51. **Wilkes JJ, Bonen A, Bell RC.** A modified high-fat diet induces insulin resistance in rat skeletal muscle but not adipocytes. *Am J Physiol Endocrinol Metab* 275: E679–E686, 1998.
52. **Willson TM, Wahli W.** Peroxisome proliferator-activated receptor agonists. *Curr Opin Chem Biol* 1: 235–241, 1997.
53. **Zierath JR, Houseknecht KL, Gnudi L, Kahn BB.** High-fat feeding impairs insulin-stimulated GLUT4 recruitment via an early insulin-signaling defect. *Diabetes* 46: 215–223, 1997.