Ghrelin Requires p53 to Stimulate Lipid Storage in Fat and Liver

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Ghrelin, a stomach-derived peptide, stimulates feeding behavior and adiposity. For its orexigenic action, ghrelin triggers a central SIRT1/p53/AMPK pathway. The tumor suppressor p53 also plays an important role in white adipose tissue (WAT), where it is up-regulated in the adipocytes of obese mice. It is not known, however, whether p53 has any role in mediating the peripheral action of ghrelin. In the present study, chronic peripheral ghrelin treatment resulted in increased body weight and fat-mass gain in wild-type mice. Correspondingly, mRNA levels of several adipogenic and fat-storage-promoting enzymes were up-regulated in WAT, whereas hepatic triglyceride content and lipogenic enzymes were also increased in wild-type mice following ghrelin treatment. In contrast, mice lacking p53 failed to respond to ghrelin treatment, with their body weight, fat mass, and adipocyte and hepatic metabolism remaining unchanged. Thus, our results show that p53 is necessary for the actions of ghrelin on WAT and liver, leading to changes in expression levels of lipogenic and adipogenic genes, and modifying body weight. (*Endocrinology* 154: 3671–3679, 2013)

Ghrelin is the only known endogenous peptidic hormone that stimulates feeding (1) and adiposity (2–4). An increase in fat mass is observed after ghrelin infusion, either centrally (3, 5) or peripherally (2). Correspondingly, ghrelin ameliorates cachexia due to a range of pathophysiological conditions like cancer or anorexia nervosa, in both humans and animal models (6–9). Although the mechanisms responsible are far from being understood, in vitro studies demonstrate that ghrelin stimulates the differentiation of rat preadipocytes, antagonizes isoproterenol-induced lipolysis (10) and stimulates lipid accumulation as well as TNFα-induced apoptosis in human visceral adipocytes (11, 12). In addition to its direct actions on white adipose tissue (WAT), chronic systemic infusion of ghrelin induces hepatic steatosis, increasing lipid droplet number and triacylglycerol content by a growth-hormone-secretagogue-receptor-1a (GHS-R1a)-dependent mechanism (13). Similarly, chronic central infusion of ghrelin also favors hepatic lipid storage and reduces lipid mobilization (5). Notably, the anabolic effects of ghrelin are, at least partially, independent of its hyperphagic effect. For instance, chronic central infusion of ghrelin in diet-induced obese (DIO) rats increases adiposity and gene expression of lipogenic enzymes in WAT even though food intake remains unchanged (14), due to DIO ghrelin resistance in arcuate NPY/AgRP neurons (15).

p53 is a tumor suppressor gene that, in addition to its biological actions in cancer, plays an important role in

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Abbreviations: AMPK, AMP-activated protein kinase; CEBPα, CCAAT/enhancer-binding protein α; DIO, diet-induced obese; FAS, fatty acid synthase; GHS-R1a, growth hormone secretagogue receptor 1a; KO, knockout; LPL, lipoprotein lipase; SREBP1c, sterol regulatory element binding protein 1c; TG, triglyceride; WAT, white adipose tissue; WT, wild type.

several metabolically relevant tissues including adipose tissue (16). p53 is highly induced in white adipocytes of leptin-deficient mice (17) and of DIO mice (18). Disruption of p53 in leptin-deficient mice can partially restore expression of lipogenic enzymes, whereas p53 overexpression suppresses expression of lipogenic genes, suggesting that activation of p53 decreases fat storage in adipocytes (17). p53 expression in WAT is also crucially involved in the development of insulin resistance, as demonstrated by the fact that inhibition of p53 activity in WAT markedly improves insulin sensitivity in mice (18, 19). Conversely, up-regulation of p53 in adipose tissue causes an inflammatory response that leads to insulin resistance (18).

In addition to its functions in adipocyte metabolism, central p53 also plays an important metabolic role in mediating ghrelin-induced food intake (20). In the present study, we therefore aimed to determine whether p53 is essential for the peripheral actions of ghrelin on lipid metabolism in WAT and liver.

Materials and Methods

Animal models

p53-null (8–10 wk old, males and females, mixed background C57BL/6J and 129/Sv) mice were used, as previously described (21). Homozygous wild-type (WT) and knockout (KO) mice were derived from heterozygous matings, and in each experiment only littermate WT and KO animals were compared. Animals were kept on a 12-hour light, 12-hour dark cycle, at 22°C, with free access to tap water. Animals were treated and killed before any sign of morbidity due to tumor development occurred. Animals were killed by decapitation between 10:00 and 12:00 hours. Animal experiments were conducted in accordance with the standards approved by the Faculty Animal Committee at the University of Santiago de Compostela, and in agreement with the Rules of Laboratory Animal Care and International Law on Animal Experimentation.

Chronic ghrelin treatment

We peripherally administered ghrelin during a 1-week period (30 μg/d, daily ip injection at 9 AM) to WT and p53-null mice (n = 7–8 per group). Food intake, body weight, body composition, and adipocyte and hepatic metabolism were assessed. Body composition was measured in injected mice using nuclear magnetic resonance (Whole Body Composition Analyzer; EchoMRI, Houston, Texas), both before ghrelin treatment and following the treatment period but before killing. On day 7, 2 hours after the last injection, mice were killed and tissues were collected and frozen at −80°C until further analysis.

Quantitative RT-PCR

RNA was extracted using Trizol reagent (Invitrogen, Carlsbad, California) according to the manufacturer's instructions. Two micrograms of total RNA was used for each RT reaction, and cDNA synthesis was performed using the SuperScript First-Strand Synthesis System (Invitrogen) and random primers as previously described (22). Negative control reactions, containing all reagents except the sample, were used to ensure specificity of the PCR amplification. For analysis of gene expression, we performed real-time RT-PCR assays using a fluorescent temperature cycler (TaqMan; Applied Biosystems, Foster City, California) following the manufacturer's instructions (22, 23). For each RT reaction, 500 ng total RNA was used. The PCR cycling conditions included an initial denaturation at 50°C for 10 minutes followed by 40 cycles at 95°C for 15 seconds and 60°C for 1 minute. The oligonucleotide-specific primers are shown in Supplemental Table 1, published on The Endocrine Society's Journals Online web site at http://endo.endojournals.org. For analysis of the data, the input value of gene expression was standardized to the 18S value for the sample group and expressed as a comparison with the average value for the control group.

Hepatic triglyceride content

The extraction procedure for tissue triglyceride (TG) was adapted from methods described previously (24). Liver tissue (approximately 200 mg) was homogenized for 2 minutes in ice-cold chloroform-methanol (2:1 vol:vol). Initial extraction of TG was carried out by 5-hour shaking at room temperature. For phase separation, H_2SO_4 was added; samples were centrifuged, and the organic lower layer was collected. The solvent was dried using a Speed Vac and the organic residue was redissolved in chloroform. TG content of each sample was measured in duplicate after evaporation of the organic solvent using an enzymatic method (Randox Laboratories Ltd, Crumlin, United Kingdom).

Effect of ghrelin on adipose tissue explants

Perigonadal adipose tissue explants were prepared by cutting samples into 5-mg portions, which were subsequently incubated for 30 minutes in PBS supplemented with 2% BSA. After a brief centrifugation (400*g*, 30 s), samples were incubated in adipocyte medium (DMEM/F12 [1:1]; Gibco, Invitrogen, Carlsbad, California) containing 17.5 mM glucose, 121 mM NaCl, 4 mM KCl, 1 mM $CaCl_2$, 25 mM HEPES, 2.4 mM sodium bicarbonate, 2% BSA, 8 mM biotin, 18 mM panthotenate, 100 mM ascorbate and antibiotic-antimycotic) for 1 hour at 37°C. Thereafter, 10^{-8} M ghrelin was added (25) and tissue explants were incubated for either 5 minutes (for the analysis of the phosphorylation rate of ERK and AMP-activated protein kinase [AMPK]) or 12 or 24 hours (for gene expression measurements) at 37°C. At the end of the experimental treatments, tissue explants were collected, washed, and processed for RNA or protein extraction as indicated below.

Western blotting

Western blotting was performed as previously described (20, 26). Briefly, total protein lysates from liver (20 μg) were subjected to SDS-PAGE, electrotransferred onto a polyvinylidene difluoride membrane, and probed with the following antibodies: ACC, phospho-ACC-Ser79 (pACC), AMPKα1 and AMPKα2 (Upstate, Lake Placid, New York); phospho-AMPK-Thr172 (pAMPK; Cell Signaling, Danvers, Massachusetts); phospho-p44/42 MAPK (Erk1/2) (Thr202/Tyr204) and Erk1/2 were from Cell Signaling Technology, Inc (Danvers, Massachusetts); β-actin (Sigma-Aldrich, St Louis, Missouri); CPT1, FAS (H-300), and LPL (H-53) (Santa Cruz Biotechnology, Santa Cruz, California).

For protein detection, we used horseradish peroxidase–conjugated secondary antibodies (Dako, Glostrup, Denmark) and chemiluminescence (Pierce ECL Western Blotting Substrate; Thermo Scientific, Rockford, Illinois). Membranes were then exposed to X-ray film (Super RX, Fuji Medical X-Ray Film; Fujifilm, Tokyo, Japan) and developed with developer and fixing liquids (AGFA, Mortsel, Belgium) under appropriate dark-room conditions. Protein levels were normalized to β-actin for each sample.

Levels of serum metabolites and hormones

Serum free-fatty-acid concentrations were determined using a kit from Wako (Richmond, Virginia); TG and cholesterol were determined using kits from Randox Laboratories Ltd. Serum insulin levels were measured using a previously described RIA technique (27). Glucose levels were measured an Accucheck glucometer (Roche, Indianapolis, Indiana) and serum corticosterone levels were measured using a corticosterone ELISA kit (Enzo Life Sciences, Telluride, Colorado) following manufactured instructions.

Data analysis and statistical tests

Values were plotted as the mean ± SEM for each genotype. Seven to eight animals per group were used for all analyses. Statistical analysis was performed using a two-way ANOVA followed by a Bonferroni's post test. R version 2.15.1. was used for data analysis. A P value less than .05 was considered statistically significant.

Results

Lack of p53 blunts chronic ghrelin-induced gains in body weight and fat mass

To assess the relevance of p53 for the action of chronic ghrelin on body weight and adiposity, we treated WT and p53 KO mice with ghrelin (30 μg/day, daily ip) for 1 week. This chronic peripheral ghrelin treatment did not modify food intake in either WT mice or mice lacking p53 (Figure 1A). Chronically administered ghrelin led to an increase in percentage body weight in WT mice, although a two-way ANOVA failed to give a significant interaction between ghrelin and p53 ($P = .25$, F = 1.31, $df = 1$) (Figure 1B). However, if an unpaired t test is used to compare only saline-treated vs ghrelin-treated WT animals, the difference was statistically significant ($P < .05$). In mice lacking p53 ghrelin failed to cause any change in body weight

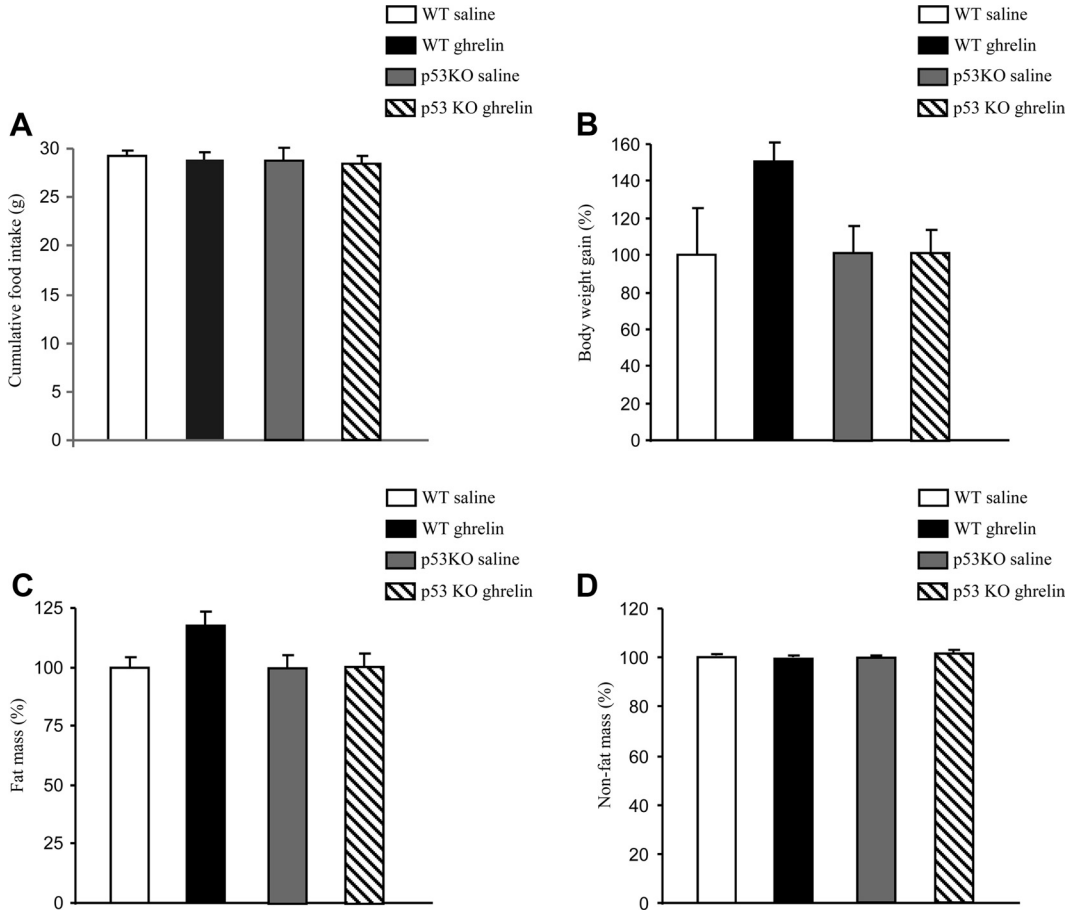

Figure 1. Effect of a 7-day ip ghrelin injection (30 μg/d) on cumulative food intake (A), percentage of body weight gain (B), percentage of total fat mass (C), and percentage of total nonfat mass (D) in WT and p53 KO mice. Values are mean ± SEM of 7–8 animals per group. Two-way ANOVA was used as statistical test.

Table 1. Body Weight (BW), Fat Mass, and Lean Mass of WT and p53-deficient Mice (KO) in Untreated Animals Fed a Standard Diet

	WT	P53 KO
BW, g	22.11 ± 1.18	20.59 ± 0.98
Fat mass, g	1.88 ± 0.09	1.98 ± 0.09
Lean mass, g	18.92 ± 0.93	17.67 ± 0.89
Fat mass, %	8.5 ± 0.34	9.67 ± 0.47
Lean mass, %	85.16 ± 1.4	85.11 ± 0.77

(Figure 1B). The increased body weight in ghrelin-treated WT mice was consistent with a gain in percentage fat mass, although again, there was not an interaction between ghrelin and p53 ($P = .07$, F = 3.38, $df = 1$) (Figure 1C), but an unpaired t test comparing only saline-treated vs ghrelin-treated WT animals showed significant differences. Fat mass was unchanged between vehicle-treated and ghrelin-treated p53 KO mice (Figure 1C). Finally, we did not observe changes in the percentage of lean mass after chronic peripheral ghrelin treatment in either WT or p53 KO mice (Figure 1D). Importantly, the body weight, food intake, and body composition was very similar between WT and p53-deficient mice before starting the ghrelin treatment (Table 1). Chronic treatment with ghrelin was associated with a positive energy balance without any significant effects on serum glucose, insulin, TG, cholesterol, free fatty acid, or corticosterone levels (Table 2).

Lack of p53 blocks the lipogenic effect of chronic ghrelin in WAT

We next assessed the expression of several key enzymes involved in the regulation of lipid metabolism in WAT. A two-way ANOVA showed that there was an interaction between ghrelin treatment and the lack of p53 for the following genes: sterol regulatory element binding protein 1c (SREBP1c) ($P = .0014$, F = 13.54, $df = 1$), fatty acid synthase (FAS) ($P = .0106$, F = 7.80, $df = 1$), lipoprotein lipase (LPL) ($P = .002$, F = 11.46, $df = 1$), and CCAAT/enhancer-binding protein α (CEBPα) ($P = .033$, F = 5.16, $df = 1$). In these genes, we then performed a Bonferroni's post test as represented in Figure 2A. WT mice chronically treated with ip ghrelin showed a significant increase in gene expression of enzymes involved in de novo synthesis of fatty acids such as SREBP1c and FAS (Figure 2A). Ghrelin treatment also stimulated expression of LPL, which favors lipid uptake, in the WAT of WT mice (Figure 2A). In addition, we found a significant up-regulation in WAT mRNA levels of CEBPα, which activates adipogenesis, in WT mice treated with ghrelin (Figure 2A). In sharp contrast to these changes found in WT WAT, we failed to detect any significant changes in mRNA expression for the same enzymes in the WAT of p53 KO mice, following chronic ghrelin treatment (Figure 2A). These results therefore indicate that p53 is essential for the adipogenic action of ghrelin in WAT. To note, a significant increase between WT and p53-deficient mice was found in the WAT expression of LPL and SREBP1 (Figure 2A).

To test if ghrelin could modulate adipocyte metabolism directly, we incubated adipose tissue explants with saline or ghrelin (10^{-8} M). In our in vitro experiments we first confirmed the efficiency of ghrelin treatment by evaluating its ability to activate ERK and AMPK at the short term. We found that, as expected, ghrelin did increase ERK and AMPK phosphorylation rate in the samples (Figure 2B). Because our in vivo experiments represented a chronic treatment with ghrelin, we next examined the expression of genes involved in lipid metabolism and adipogenesis in the adipose tissue explants of WT mice after long-term incubation with saline or vehicle. After 12 or 24 hours, ghrelin did not cause any significant change in the expression levels of any of the genes examined when directly administered to adipose tissue explants, with the exception of ACCα that was decreased after 24 hours of exposure to the peptide (Figure 2, C and D) Thus, these findings indicate that the chronic effect of ghrelin on adipose tissue metabolism is not direct.

Lack of p53 blocks the lipogenic effect of ghrelin in liver

Next, we investigated whether the lack of p53 was modulating the effects of ghrelin specifically in WAT, or whether it was also relevant to ghrelin's action on another

Table 2. Circulating Levels of Nonesterified Fatty Acids (NEFAs), TG, Cholesterol, Glucose, Insulin, and Corticosterone in WT and p53 KO Mice, in Chronically Ghrelin-treated (GHR) and Control (SAL) Groups (7–8 animals per group)

	WT-SAL	WT-GHR	KO-SAL	KO-GHR
NEFAs, mg/dL	39.42 ± 5.66	31.37 ± 2.59	31.37 ± 2.59	25.78 ± 2.65
TG, mg/dL	7.00 ± 0.77	6.5 ± 0.37	5.1 ± 0.40	5.67 ± 0.73
Cholesterol, mg/dL	16.81 ± 0.86	20.68 ± 1.53	16.77 ± 0.83	18.50 ± 1.16
Glucose, mg/dL	14.85 ± 1.32	12.52 ± 1,08	13.08 ± 0.57	14.03 ± 0.49
Insulin, ng/mL	0.66 ± 0.15	0.73 ± 0.11	0.46 ± 0.08	0.56 ± 0.05
Corticoesterone, ng/mL	5.51 ± 1.12	5.76 ± 1.35	5.27 ± 1.49	5.36 ± 0.75

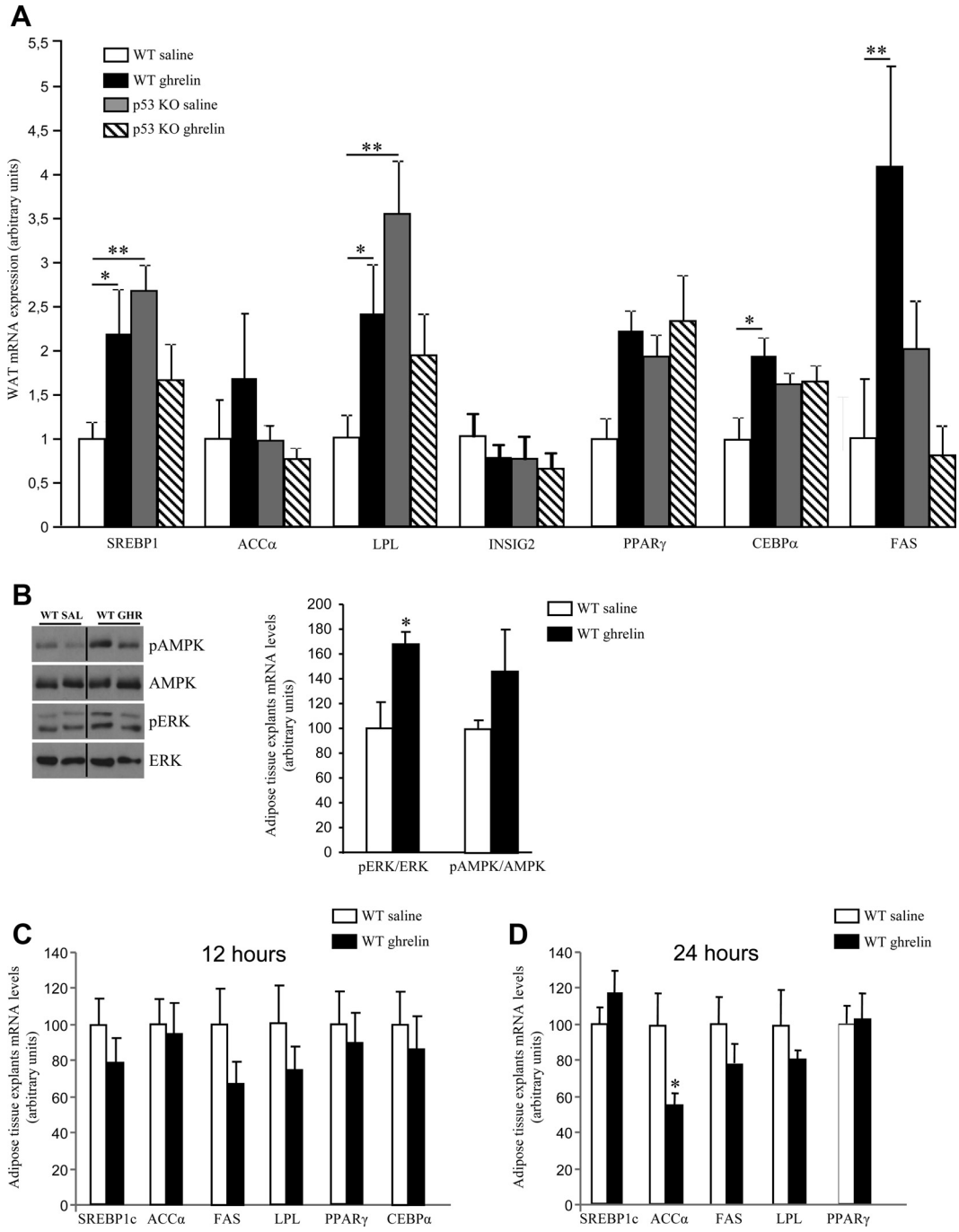

Figure 2. Effects of a 7-day ip ghrelin injection (30 μg/d) on epididymal WAT mRNA expression of SREBP1c, acetyl CoA carboxylase α (ACCα), LPL, insulin-induced gene 2 (INSIG2), peroxisome proliferator-activated receptor γ (PPARγ), CEBPα, and FAS, in WT and p53 KO mice (A). On day 7, 2 hours after the last injection, mice were killed and tissues were collected and frozen at −80°C until further analysis. Values are mean ± SEM of 7 to 8 animals per group. Two-way ANOVA followed by a Bonferroni's post test was used as statistical test. Effect of ghrelin (10^{-8} M) on WT mice adipose tissue explants on pAMPK, AMPK, pERK, ERK levels (n = 3) (B) and SREBP1c, ACCα, LPL, PPARγ, CEBPα, and FAS mRNA expression after 12 hours (n = 6) (C) and 24 hours (n = 6) (D). *, $P < .05$; **, $P < .01$.

peripheral metabolic tissue, the liver. We found that chronic peripheral treatment with ghrelin increased hepatic TG content in WT but not in p53-deficient mice (Figure 3A). To dissect the molecular underpinnings of the increased content of hepatic TG in ghrelin-treated WT mice further, we analyzed the expression profile of key enzymes involved in liver lipid metabolism. First, we found a significant interaction between ghrelin treatment and the lack of p53 for the hepatic levels of ACCα ($P = .0005$, F = 17, $df = 1$) and FAS ($P = .017$, F = 6.53, $df = 1$). In these 2 genes, we then performed a Bonferroni's post test as represented in Figure 3C. Chronic ip ghrelin mark-

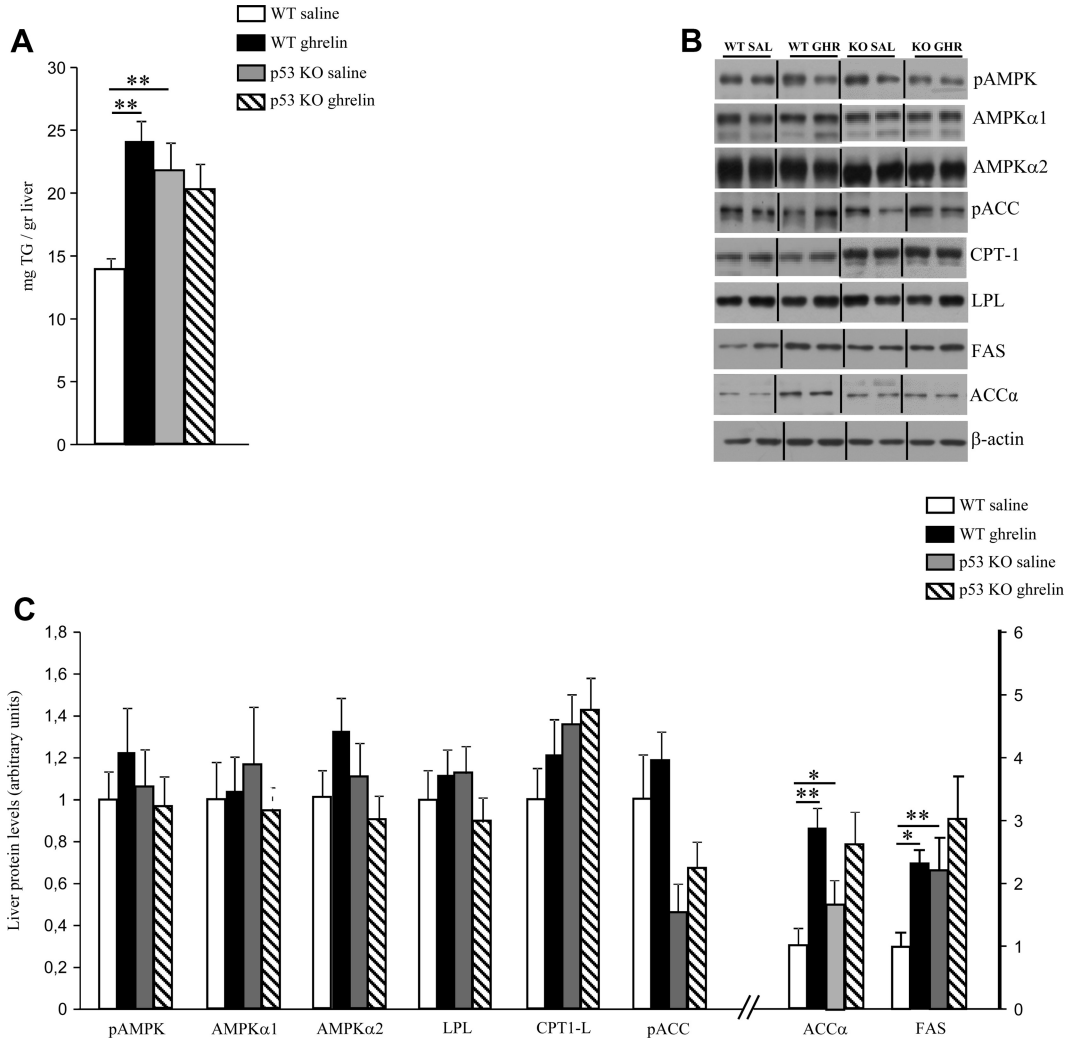

Figure 3. Effect of a 7-day ip ghrelin injection (30 μg/d) on hepatic TG content (A), and hepatic protein levels of phosphorylated AMP-activated protein kinase (pAMPK), AMPKα1, AMPKα2, phosphorylated acetyl CoA carboxylase (pACC), carnitine palmitoyltransferase-1 (CPT1), LPL, ACCα, and FAS, in WT and p53 KO mice (B and C). On day 7, 2 hours after the last injection, mice were killed and tissues were collected and frozen at −80°C until further analysis. Values are mean ± SEM of 7 to 8 animals per group. Two-way ANOVA followed by a Bonferroni's post test was used as statistical test. *, $P < .05$ **, $P < .01$.

edly increased hepatic protein levels of enzymes favoring lipid synthesis, namely ACCα and FAS, in WT mice (Figure 3, B and C), whereas the peptide had no effect on enzymes promoting fatty acid oxidation such as CPT-1. This was not the case, however, in p53-deficient mice, which did not show any significant changes in any of the proteins studied (Figure 3, B and C). Overall, these results suggest that p53 also plays a relevant role in the lipogenic action of ghrelin in the liver. To note, hepatic levels of TG, ACC, and FAS were significantly increased in p53-deficient mice in comparison to WT mice, indicating that the lack of this gene causes hepatic alterations in lipid metabolism.

Lack of p53 blocks the hypothalamic action of ghrelin on lipid metabolism

Because it is well known that short-term (2–6 h) ghrelin modulates hypothalamic AMPK-fatty acid metabolism (28, 29) through the SIRT1-p53 pathway (20), we finally assessed if the chronic peripheral injection of ghrelin was affecting hypothalamic lipid metabolism. We found a significant interaction between ghrelin treatment and the lack of p53 for the hypothalamic levels of ACCα ($P = .02$, $F = 6.30$, $df = 1$) and FAS ($P = .03$, $F = 5.11$, $df = 1$). In these 2 genes, we then performed a Bonferroni's post test as represented in Figure 3C. We found that chronic peripheral treatment with ghrelin decreased the hypothalamic protein levels of ACCα and FAS in WT mice, but not in ghrelin-treated p53-deficient mice (Figure 4). Although it was reported that acute central infusion of ghrelin increased hypothalamic pAMPK levels (28, 29), in our study we failed to detect significant changes in hypothalamic pAMPK levels after the chronic peripheral injections as reported in previous studies (30), indicating that phophorylation of this enzyme by ghrelin is time-dependent.

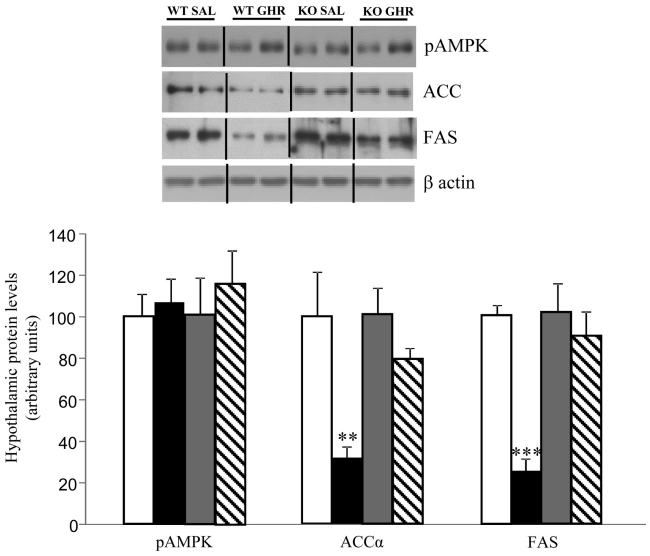

Figure 4. Effect of a 7-day ip ghrelin injection (30 μg/d) on hypothalamic protein levels of phosphorylated AMP-activated protein kinase (pAMPK), ACCα, and FAS, in WT and p53 KO mice. On day 7, 2 hours after the last injection, mice were killed and tissues were collected and frozen at −80°C until further analysis. Values are mean ± SEM of 7 to 8 animals per group. Two-way ANOVA followed by a Bonferroni's post test was used as statistical test. **, $P < .01$; ***, $P < .001$.

Overall, these results indicate that the brain p53 system might be involved in the actions of ghrelin on liver and WAT metabolism.

Discussion

Numerous reports indicate that pharmacological or genetic manipulation of the ghrelin system affects adiposity and body weight. Central and peripheral administration of ghrelin increases adiposity and body weight gain (2, 3, 5). Consistent with this, elegant studies based on genetic manipulations indicate that mice lacking ghrelin (31) or ghrelin receptors (32) accumulate less fat mass than their WT littermates when fed a high-fat diet. Moreover, ablation of the ghrelin receptor reduces adiposity during aging by regulating adipocyte metabolism (33). Although the adipogenic action of this peptidic hormone is indubitable, the molecular pathways by which ghrelin favors lipid deposition remain largely unknown. Our current data demonstrate that p53 is crucial for the adipogenic and lipogenic effect of ghrelin. These results appear to indicate that a lack of p53 abolishes the stimulation of lipid storage induced by chronically administered ghrelin in WAT and liver.

Most effects mediated by ghrelin are exerted through the GHS-R1a (34). GHS-R1a is widely expressed throughout the central nervous system and in peripheral tissues (35, 36), and although this receptor mediates both adipogenic and orexigenic actions of ghrelin, the mechanisms controlling each process are different. The evidence for this is that in DIO rats the hyperphagic effect of ghrelin disappears (14, 15) even though the gain of adiposity remains intact (14). It therefore seems likely that central and peripheral pathways controlling ghrelin-induced feeding behavior and adiposity are divergent. This hypothesis is supported by our current data that under our experimental conditions chronic peripheral treatment with ghrelin increased body weight gain and adiposity in WT mice, without any alteration of food intake.

At the hypothalamic level, activation of GHS-R1a causes a cellular response in which hypothalamic SIRT1 is activated (20, 37) to deacetylate p53; this in turn leads to increased phosphorylation of AMPK and subsequent inactivation of the de novo fatty-acid biosynthetic pathway, with concomitant activation of fatty-acid oxidation (28). These molecular events induce changes in UCP2 and reactive oxygen species (29), and up-regulation of the transcription factors Bsx (38, 39), FoxO1, and pCREB (26). The activation of these transcription factors leads to the ultimate step that includes an increased transcription rate of NPY and AgRP (40). Peripherally, however, studies addressing the molecular mechanisms by which ghrelin controls adipocyte metabolism are scarce. One in vitro study has shown that ghrelin stimulates the activity of glycerol-3-phosphate dehydrogenase in rat preadipocytes and increases PPARγ2 gene expression in primary cultured differentiated adipocytes from rats (10). In vivo reports demonstrate that ghrelin stimulates lipid deposition in WAT and liver by increasing the expression and activity of key enzymes involved in fatty acid metabolism, such as SCD1, ACCα, and FAS (3, 5). However, the molecular events following activation of GHS-R1a but before modulation of enzymes involved in lipid metabolism remain unknown.

Our in vivo observation that a lack of p53 abolishes the lipogenic action of ghrelin in WAT and liver suggests that this tumor suppressor is an important mediator of ghrelin activity not only in the brain but also in peripheral tissues. Interestingly, our in vivo study in p53-null mice shows that in the absence of p53 ghrelin fails to stimulate the expression of genes responsible for 2 functions in WAT—those that favor lipid uptake and deposition (SREBP1c, FAS, LPL) and those that promote adipocyte differentiation (PPARγ, CEBPα). Because the increased weight gain and fat mass acquitted by WT ghrelin-treated mice failed to be statistically significant when using a two-way ANOVA test, it is likely that this discrepancy will disappear, increasing the length of treatment. Our findings appear to indicate that all the biochemical changes detected in WAT

and liver are likely preceding changes in fat mass and body weight.

Another important question was whether these actions of ghrelin on WAT and liver were mediated by the brain p53 system. Although a chronic central ghrelin infusion would address this question directly, unfortunately mice lacking p53 did not withstand the surgery after several days. Nevertheless, our findings support the hypothesis that these actions might be modulated by the brain p53 system, because 1) our in vitro results indicate that adipose tissue explants incubated at long term with ghrelin did not show any alteration in the expression of lipogenic or adipogenic genes, and 2) chronic peripheral treatment with ghrelin decreased the hypothalamic protein levels of FAS, which are downstream of p53 and AMPK (20, 28, 29).

It is also important to point out that WT and mice lacking p53 fed a standard diet did not show differences in body weight, food intake, or body composition. However, they did show a significant increase in the expression of enzymes modulating lipid metabolism in WAT (SREBP1 and LPL) and liver (ACCα and FAS) as well as in hepatic TG levels. These results partially agree with previous reports indicating that p53 plays an important role in adipose tissue (18, 19). Indeed, our findings might indicate that body weight and fat mass of mice lacking p53 might respond differentially to WT when they are challenged to certain conditions such as high fat diet, but further studies will be necessary to address this issue.

In summary, we demonstrate here that p53 is essential for the peripheral actions of chronically administered ghrelin on WAT and liver, because it regulates lipogenic and adipogenic gene expression that lead ultimately to an increase in body weight.

Acknowledgments

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