Published in final edited form as:

Endocr Res. 2013 August ; 38(3): 139–150. doi:10.3109/07435800.2012.735735.

Glucose tolerance in mice is linked to the dose of the p53 transactivation domain

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Abstract

The tumor suppressor p53 has a critical role in maintenance of glucose homeostasis. Phosphorylation of Ser[18] in the transaction domain of p53 controls the expression of *Zpf385a*, a zinc finger protein that regulates adipogenesis and adipose function. Mice with a mutation in p53Ser[18] exhibit reduced *Zpf385a* expression in adipose tissue, adipose tissue-specific insulin resistance, and glucose intolerance. Mice with relative deficits in the transactivation domain of p53 exhibit similar defects in glucose homeostasis, while “Super p53” mice with an increased dosage of p53 exhibit improved glucose tolerance. These data support the role of an ATM—p53 cellular stress axis that helps combat glucose intolerance and insulin resistance and regulates glucose homeostasis.

Keywords

p53; glucose intolerance; mouse genetics

Introduction

p53 is activated by numerous stresses that impact metabolism, including atherosclerosis, trauma, hypoxia, and infections [5, 17, 20, 21, 29, 32, 34]. In addition, p53 is activated directly by glucose levels. In response to hyperglycemia, p53 is induced and phosphorylated at Ser[18], Ser[376], and Ser[390] [6] and mediates hyperglycemia-induced apoptosis in mouse blastocysts [13] and myocytes [6]. p53 transactivates genes involved in the control of

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Conflict of interest statement. The authors declare they have no conflict of interest.

glycolysis and oxidative phosphorylation [8] both *in vitro* and *in vivo*, such as the glucose transporters *Glut1* and *Glut4* [24, 31], *TIGAR* [11], and *Sco2* [16]. p53 also regulates the expression of the zinc-finger protein 385a (*Zfp385a*, also known as *hzf*), a gene involved in adipocyte function and glucose homeostasis [12].

Animal studies have also implicated p53 in metabolic control. p53 has been shown to play a role in lipid-induced insulin resistance through regulation of senescence [19]. p53 mediates autoimmune disease and the macrophage response in a streptozotocin-induced type 1 diabetes model [35]. Morever, the link of p53 with metabolic control has been established through studies with the p53 regulatory kinase ATM, which is also activated by many of the same cellular stresses as p53. ATM dysfunction is correlated with insulin resistance and defects in glucose homeostasis in both human and mice [2, 18, 22, 23]. ATM is activated by insulin and this can lead to p53Ser^{18} phosphorylation [33]. In addition, our studies have demonstrated that mice with defects in p53Ser^{18} phosphorylation, an ATM target, develop insulin resistance [1]. However, important mechanistic questions remain about how p53 contributes to systemic glucose homeostasis and what the specific role of the transcriptional activation domain in this process might be.

We have taken advantage of a unique strain of p53Ser^{18} mutant mice in which the expression of *Zfp385a* is reduced specifically in adipose tissue to explore the connection between p53 transcriptional control of metabolic genes and insulin sensitivity. We utilized two additional strains of p53 mutant mice to test the hypothesis that glucose homeostasis depends on the level of p53 activity and the functional capacity of the transactivation domain encoded in the first 40 amino acids of the protein. These studies establish a pivotal role for p53 in metabolic homeostasis.

Materials and Methods

Mouse strains and diet information

The methods employed for the generation and genotyping of $p53^{S18A}$ mice [26], $p53^{-/-}$ mice [4], *p44Tg* mice [15], and $p53^{super}$ mice [7] have been reported previously. $p53^{S18A}$ mice were backcrossed ten generations on the C57BL/6 genetic background (Jackson Labs). $p53^{super}$ mice were on a C57BL/6 background. *p44Tg* mice were on an ICR/B6SJL mixed background and mated once to C57BL/6, and wildtype and *p44Tg* mice were established from intercrosses from the F1 generation. Cohorts of age-matched male mice were established for analysis. The mice were maintained on a standard chow diet. All mice were housed in specific pathogen-free facilities accredited by the American Association for Laboratory Animal Care. The Institutional Animal Care and Use Committees of the University of Massachusetts Medical School approved all studies using animals. Importantly, all animals used in our experiments were determined to be tumor-free.

Glucose and insulin tolerance tests

Glucose homeostasis was examined using a glucose tolerance test (GTT) and an insulin tolerance test (ITT) with methods described previously [1]. Mice were fasted overnight and challenged by intraperitoneal administration of glucose (1g/kg body weight, IMS) or insulin

(0.75 units/kg body weight). Blood glucose was measured with an Ascenzia Breeze 2 glucometer (Bayer) and blood insulin was measured by ELISA (Luminex 200, Millipore). Due to their shortened life span, we conducted experiments on *p44Tg* male mice at 4 months of age rather than the 6–7 month age used for p53S18AB6 mice.

Hyperinsulinemic-euglycemic clamp studies

The clamp studies were performed at the University of Massachusetts Mouse Metabolic Phenotyping Center. Whole body fat and lean mass were non-invasively measured using ^{1}H-MRS (Echo Medical Systems). Following an overnight fast, a 2-hr hyperinsulinemic-euglycemic clamp was conducted in conscious mice with a primed and continuous infusion of human insulin (150 mU/kg body weight priming followed by 2.5 mU/kg/min; Humulin; Eli Lilly), and 20% glucose was infused at variable rates to maintain euglycemia [14]. Whole body glucose turnover was assessed with a continuous infusion of [3-^{3}H]glucose (PerkinElmer) and 2-deoxy-D-[1-^{14}C]glucose (PerkinElmer) was administered as a bolus (10 μCi) at 75 min after the start of clamp to measure insulin-stimulated glucose uptake in individual organs. At the end of the clamp, mice were anesthetized, and tissues were taken for biochemical analysis [14].

Analysis of tissue sections

Upon necropsy gross organ analysis was performed. In addition, samples of liver, spleen, thymus, lymph nodes, kidney, heart, pancreas, fat and muscle were removed. Histology was performed using tissue fixed in 10% formalin for 24h, dehydrated and embedded in paraffin. Sections (7μm) were cut, stained using hematoxylin and eosin (American Master Tech Scientific), and examined by a board-certified veterinary pathologist.

Insulin treatment and immunoblot analysis

Mice were fasted overnight and injected with 1.5 U of insulin and tissues were harvested 30 min later. Tissues were homogenized in triton lysis buffer [20 mM Tris (pH 7.4), 1% Triton X-100,10% glycerol, 137 mM NaCl, 2 mM EDTA, 25 mM β-glycerophosphate, 1 mM sodium orthovanadate, 1 mM phenylmethylsulfonyl fluoride, and 10 μg/mL of aprotinin and leupeptin]. Tissue extracts (50 μg) were examined by immunoblot analysis using antibodies to Akt, phospho-Ser^{473} Akt and phospho-Thr^{308} Akt (Cell Signaling).

Cytokine analysis

Cytokines in plasma were measured by multiplexed ELISA using a Luminex 200 machine (Millipore) and serum mouse adipokine, adiponectin, and cytokine kits (Millipore).

Blood lipid analysis

Triglycerides were measured using the CardioCheck meter and Triglyceride Strips (Polyment Technoogy Systems, Indianapolis, IN).

RNA preparation and analysis

RNA was prepared from tissues collected in RNA-later (Ambion, Life Technologies, Grand Island, NY) and snap frozen in liquid nitrogen. Total RNA was prepared with RNeasy kits

(Qiagen, Valencia, CA) following manufacturer's instructions. The purified RNA was subjected to an additional DNAse treatment (Ambion, Life Technologies, Grand Island, NY) to ensure removal of contaminating genomic DNA prior to final column purification. The relative expression of mRNA was examined by quantitative PCR analysis. cDNA was prepared using Superscript III (Invitrogen, Life Technologies, Grand Island, NY) with random hexamers and 0.5μg - 1μg of RNA per tissue. Quantitative real-time PCR was performed on a Biorad iCycler using SyBr Green master mix (Biorad, Hercules, CA). The primer sequences for the murine genes were: *Gapdh* (5'-CTTCACCACCATGGAGAAGGC-3'; 5'-GGCATGGACTGTGGTCAT-3'); *p53* (5'-TGAAACGCCGACCTATCCTTA-3'; 5'-GGCACAAACACGAACCTCAAA-3'); *Mdm2* (5'-TGACACCAGAGCTTAGTCCTG-3'; 5'-GCGTCTCGTAACGAATAAGGC-3'); *Zfp385a* (5'-ACATTGAGCACCGCTATGTCT-3'; 5'-CTCTCTTGGATGAGGGTCTGATA-3'); *Sesn1* (5'-GTGGACCCAGAACGAGATGACGTGGC -3'; 5'-GACACTGTGGAAGGCAGCTATGTGC -3'); *Sesn2* (5'-TCCGAGTGCCATTCCGAGAT-3'; 5'-TCCGGGTGTAGACCCATCAC-3'); *Sesn3* (5'-GCGAGGAGAAGAACATTTGCC-3'; 5'-CCAAACATACAGTGAACATAGT-3'). All samples were examined in triplicate and values were normalized for baseline expression and for expression of *Gapdh*. Calculations of values were made using the ΔΔCt method. Statistical significance was calculated using CT values.

Data analysis

To calculate statistical changes in metabolic parameters, statistically significant differences ($P < 0.050$) between groups were examined using the two-tailed Student's T-test. Microsoft Excel was used for statistical calculations.

RESULTS

$p53^{S18A(B6)}$ mice have reduced glucose tolerance, but normal insulin sensitivity

We previously reported that mice defective in phosphorylated p53Ser^{18} (the equivalent of Ser^{15} in human p53) develop both glucose intolerance and insulin resistance [1]. In addition, we reported p53Ser^{18}-dependent regulation of the anti-oxidant sestrin genes (*sesn1 and sesn2*) and adipogenesis regulator *Zfp385* (also known as *hzf*) [1]. As shown in Fig. 1A, we have subsequently discovered that a sub-strain of p53Ser^{18} mutant mice on a C57Bl/6 genetic background, which we have designated as $p53^{S18A(B6)}$ mice, exhibit decreased expression of *Zfp385a*, but not *sesn1* or *sesn2,* selectively in adipose tissue. By comparison, expression levels of *Zfp385a*, *sesn1* and *sesn2* were the same in other tissues from wild type and $p53^{S18A}$(B6) mice, as shown for liver in Fig. 1B.

We analyzed glucose metabolism in $p53^{S18A(B6)}$ animals by performing glucose and insulin tolerance tests on tumor-free animals. By 6 months of age, $p53^{S18A(B6)}$ mice on a normal chow diet were glucose intolerant (Fig. 2A). Serum glucose levels were significantly elevated in $p53^{S18A(B6)}$ mice compared to wild type mice at 30 and 60 min following intraperitoneal glucose injection. At 30 min, average peak glucose levels for $p53^{S18A(B6)}$ males were measured to be 337 ± 17 mg/dL compared with 285 ± 19 mg/dL in wild type

males. At 60 min, average peak glucose levels for $p53^{S18A(B6)}$ males were 299 ± 22 mg/dL compared with 222 ± 18 mg/dL in wild type mice. And at 150 min, the $p53^{S18A(B6)}$ mice had an elevated mean glucose compared to wild type (188 ± 15 and 135 ± 5 mg/dL, respectively). At the same time, we did not observe overt insulin resistance during an insulin tolerance test (data not shown).

To pursue the issue of insensitivity further, we examined other parameters of metabolic function. Obesity contributes to defects in glucose homeostasis, but in this sub-strain we did not observe a significant difference from wild type in body weight (Fig. 2B) or food consumption (data not shown). We did find a significantly increased level of serum insulin in $p53^{S18A(B6)}$ animals compared to wild type animals (Fig. 2C), which, however, was not associated with insulin resistance, as shown by the insulin tolerance test. Furthermore, although there was no significant increases in circulating IL-6 (Fig. 2D), TNF α levels were elevated (Fig. 2E). Elevated levels of TNFα are associated with insulin resistance and obesity [3, 30]. We also performed a hyperinsulinemic-euglycemic clamp study to examine insulin sensitivity in conscious mice. We examined five 6 month-old animals of each genotype with similar body weights. During the euglycemic clamps, plasma glucose levels were similarly maintained at 140–160 mg/dl in both groups of mice (Fig. S1A). The steady-state glucose infusion rate during the clamp was similar in $p53^{S18A(B6)}$ mice and wild type mice (Fig. S1B). Insulin-stimulated whole body glucose turnover was modestly reduced in wild type compared to $p53^{S18A(B6)}$ mice (Fig. S1C), while whole body glycolysis was slightly reduced in $p53^{S18A(B6)}$ mice compared to wild type (Fig. S1D).

To summarize the results of these metabolic tests, we found that $p53^{S18A(B6)}$ mice have reduced glucose tolerance, but normal whole body insulin sensitivity, in contrast to $p53^{S18A}$ mice, which exhibit both reduced glucose tolerance and reduced insulin sensitivity [1]. One possible explanation might be that glucose intolerance is due to insulin resistance in one, but not all, insulin-sensitive tissues in the $p53^{S18A(B6)}$ sub-line.

$p53^{S18A(B6)}$ mice have impaired function and selective insulin resistance in adipose tissue

To test this hypothesis, we analyzed insulin sensitivity of peripheral tissues in $p53^{S18A(B6)}$ mice. We found no differences in skeletal muscle insulin sensitivity (Fig. S1E), or in insulin-stimulated whole body lipid and glycogen synthesis, basal hepatic glucose production (HGP), and insulin-stimulated HGP (Fig. S1F, G, and H). Thus, these animals did not exhibit either hepatic or muscle insulin resistance. In contrast, insulin-stimulated glucose uptake by white adipose tissue during the clamp was markedly reduced in $p53^{S18A(B6)}$ mice (Fig. 3A), indicating that insulin resistance was specific to adipose tissue.

To determine if insulin resistance could be due to adipose tissue dysfunction, we compared white adipose tissue in $p53^{S18A(B6)}$ and wild type mice. Histological analysis revealed that there were no morphological differences, although adipose tissue from $p53^{S18A(B6)}$ animals appeared to be more uniform in size (Fig. 3B). Fasting blood triglyceride levels were similar in $p53^{S18A(B6)}$ mice and wild type mice (data not shown). Circulating plasma leptin levels were also similar (Fig. 3C), but adiponectin levels were lower in $p53^{S18A(B6)}$ animals compared to wild type (Fig. 3D). In contrast, there was no difference in resistin levels (Fig.

3E). Together, these data demonstrate that *p53*$^{S18A(B6)}$ mice exhibit selective insulin resistance in adipose tissue, which is likely due to defects in adipose tissue function.

p44Tg mice have reduced glucose tolerance and defects in adipose tissue

To begin to understand the relationship between the Ser^{18} mutation, which affects the transactivation domain of p53, and glucose sensitivity and adipose tissue, we analyzed another line of p53 mutant mice. *p44Tg* mice have an extra (ectopic) allele of *p53* that codes for Δ40p53, a p53 isoform lacking the transactivation domain. Expression of both endogenous and ectopic *p53* genes results in an imbalance in p53 and Δ40p53 such that there is a higher ratio of Δ40p53 to full-length p53 and a skewed contribution of Δ40p53 to p53 tetramers. The effect can be compared to effect of the Ser^{18} mutation on the activity of the p53 transcription factor [27]. If the physiological phenotype of *p53*$^{S18A(B6)}$ mice is linked to the transactivation domain of p53, then *p44Tg* might exhibit similar changes. To test this hypothesis, we subjected *p44Tg* mice to a glucose tolerance test and found they exhibited significantly increased glucose levels at 15 and 30 minutes post-injection (Fig. 4A). Thus, like *p53*$^{S18A(B6)}$ mice, *p44Tg* mice are glucose intolerant. In contrast to *p53*$^{S18A(B6)}$ mice, however, *p44Tg* mice exhibited increased, rather than decreased, sensitivity during an insulin tolerance test (Fig. 4B) and lower than normal triglyceride levels in both the fed and fasted states (Fig. 4C). These data are consistent with published results, demonstrating that, despite a defect in beta cell mass and significantly reduced insulin levels, young *p44Tg* mice are not hyperglycemic [9]. They do, however, have defects in adipose tissue such that the expression of genes that together regulate adipogenesis (*CEBPα*, *CEBPδ*), fatty acid storage (*PPARγ, PGC-1, AP2*), and systemic glucose metabolism (adiponectin and leptin) are significantly reduced. As shown in Fig. 4D, the levels found in adipose tissue (epididymal fat pad) from *p44Tg* mice (open bars) were lower than normal adipose tissue (black bars) even at a young age. The level of *UCP-1*, on the other hand, which is not involved in adipocyte generation or function, was not different. Thus, mutations that impair the ability of p53 to transactivate (or transrepress) target genes, either by interfering with phosphorylation of critical sites (Ser^{18}) or altering the stoichiometry of p53 tetramers with or without the transactivation domain, cause decreased glucose sensitivity that could be attributed at least in part to deficits in adipose tissue.

Increased *p53* dosage improves glucose tolerance

To further confirm that p53Ser^{18} regulates metabolism, we examined glucose homeostasis in “Super p53” mice [7], which have an additional dose of *p53* carried on a BAC transgene, resulting in greater p53Ser^{18}-dependent gene expression. We observed a significant improvement in glucose response in p53^{super} compared to wildtype (WT) mice, as shown by glucose tolerance (Fig. 5A) and insulin tolerance (Fig. 5B). These studies further highlight the importance of p53 pathway in regulation of glucose homeostasis.

Discussion

p53 is a tumor suppressor and regulator of cellular stress. Various cellular stresses connected with defective metabolism, including atherosclerosis, trauma, hypoxia, and infections, are associated with p53 activation and phosphorylation of p53 at Ser^{18} [5, 17, 20, 21, 29, 32,

34]. ATM, the kinase that phosphorylates p53Ser^{18}, is also activated by these same stresses. In addition, patients with mutations in the *ATM* gene, which cause ataxia telangiectasia, exhibit an increased risk of developing insulin resistance and type 2 diabetes [2], and loss of *Atm* in mice has been implicated in insulin resistance and metabolic disease [25]. Furthermore, we have demonstrated a defect in glucose homeostasis in animals expressing a mutation at p53Ser^{18}, an ATM target site [1].

p53Ser^{18} mutant animals (*p53^{S18A}* mice) on a mixed genetic background exhibited increased oxidative stress, likely due to loss of sestrin gene expression in the liver, which contributed to defects in glucose homeostasis [1]. However, the animals also exhibited deficits in *Zfp385a* expression and in levels of circulating adipokines. We subsequently identified a sub-strain of *p53^{S18A}* mice that exhibited adipose-specific loss of p53Ser^{18}-dependent *Zfp385a* expression. We report in this study that *p53^{S18A}* animals on a C57BL/6 background [*p53$^{S18A(B6)}$* mice] had a significant reduction in *Zfp385a* expression in adipose tissue, but no changes in *sesn* expression levels in the liver (Fig. 1A, B). This enabled us to study the effects of loss of p53Ser^{18}-dependent expression of *Zfp385a* in adipose tissue.

p53$^{S18A(B6)}$ mice exhibited glucose intolerance at 6 months of age (Fig. 2), confirming our previous findings that the ATM phosphorylation site on murine p53Ser^{18} exerts a protective role in maintaining glucose homeostasis [1]. The animals exhibited increased circulating plasma insulin levels, which could have indicated insulin resistance, but none was observed in a serum insulin tolerance test (data not shown). However, clamp studies did identify insulin resistance specifically in adipose tissue (Fig. 3A), with none observed in liver or muscle, suggesting a defect in adipose tissue function. Consistent with this, we found lower levels of circulating adiponectin. Although the mechanism is unclear, we propose that it is the loss of *Zfp385a* that is interfering with adipose tissue function, perhaps by altering the translation of *CEBPα*, as suggested in a previous report [12]. The insulin resistance in adipose tissue, decreased *Zfp385a* expression in white adipose tissue, decreased circulating levels of adiponectin levels, as well as increased TNFα levels suggest that the defect in glucose tolerance in *p53$^{S18A(B6)}$* mice is due to defects in adipose tissue function. This is an important finding and confirms the role of adipose tissue in overall glucose homeostasis.

To test our hypothesis that p53 positively regulates glucose homeostasis, we analyzed glucose handling in mice with an extra dose of p53, which we predicated would lead to improved glucose homeostasis. "Super p53 mice" (*p53^{super}* mice) have an ectopic *p53* locus in which p53 expression is under normal genomic control. These mice exhibited significantly improved glucose homeostasis at 6 months of age (Fig. 5). Although the mechanism for this improved glucose tolerance is not clear, it has been reported that Super p53 mice exhibit increased *sestrin* gene expression, suggesting that they might have decreased oxidative stress. Alternatively, they might have improved adipose tissue function, which could contribute to improved glucose homeostasis.

We also examined glucose handling in a line of mutant p53 mice in which an ectopic allele of *p53* encoding a specific p53 isoform (Δ40p53) alters the balance between this isoform and full-length p53. Δ40p53 is identical to p53 except that it is missing the first 40 amino acids, including Ser^{18}. Thus, mice with an extra dose of Δ40p53 (*p44Tg* mice) compared to wild

type mice have a reduced amount of Ser^{18}, whereas Super p53 mice have an increased amount of Ser^{18}. Consistent with these Ser^{18} dosage differences, we confirmed previously published results [8] that *p44Tg* mice exhibit decreased glucose tolerance (Fig. 4). However, we observed improved insulin sensitivity in these same mice, which would suggest that the tissues are not insulin resistant but that some aspect of insulin production or secretion is defective. Along these lines, a recent study demonstrated that defects in glucose tolerance were coupled to decreased beta cell mass and lower insulin levels in *p44Tg* mice [9].

Because Δ40p53 interferes with the function of the p53 transcription factor [8, 12, and [28]], the extra dose of Δ40p53 in *p44Tg* mice could easily lead to changes in the expression of p53 target genes, such as *Zfp385a*, and perturbation of glucose homeostasis. This could explain why the expression of an array of genes involved in adipogenesis and adipocyte-specific utilization of glucose was significantly lower in white adipose tissue from *p44Tg* mice compared to that from normal mice. In all p53 models, changes in glucose tolerance (decreased in $p53^{S18A(B6)}$ and *p44Tg* mice and increased in Super p53 mice) did not occur until the mice were middle-aged. Thus, it is unlikely that p53 exerts a direct affect on insulin signaling *per se*. However, altered transcription of other p53 target genes that regulate metabolism, such as *Glut-1* or *-4* [24, 31], *TIGAR* [11], or *Sco2* [16], could contribute substantially to the metabolic phenotype of these various mouse strains. The results presented in this paper link functional deficits in the transactivation domain of the p53 protein, which stem from completely different *p53* mutations in isolated strains of p53 mutant mice, to adipose tissue-specific defects that contribute to impaired glucose handling. How these defects in relatively young animals lead to serious consequences, such as diabetes, when they are older will be an important question to pursue.

Conclusions

We have utilized a mouse model wherein $p53Ser^{18}$ is no longer phosphorylated to study the contribution of p53 activation to glucose homeostasis. We have identified animals that exhibit adipose, but not liver, specific defects of $p53Ser^{18}$-dependent transcription, such as *Zfp385a*. These animals demonstrate glucose intolerance. In addition, we examined glucose homeostasis in two additional models of p53 function, one in which p53 transcriptional activity is perturbed and another with increased p53 activity. Mice with an increased dosage of *p53* exhibited better than normal glucose homeostasis. The results of this study support a model in which normal p53 function acts as a barrier to glucose intolerance and insulin resistance.

Acknowledgments

We thank Roger Davis for critical reading of the manuscript, Judy O'Reilly for invaluable technical assistance, David Garlick for histology analysis, and Ceren Acer and Charissa Cottonham for assistance with real-time PCR analysis. We thank Dr. Serrano for the $p53^{super}$ mice. C.D. was supported by a Fuller Fellowship from the American Cancer Society. These studies were supported by a Pilot & Feasibility grant from the UMASS Diabetes Endocrinology Research Center (NIH P30 DK32520) (to H.K.S.), a Worcester Foundation Annual Research Award from the Worcester Foundation for Biomedical Research (to H.K.S.) and by grants from the NIH (DK80756 to J.K.K. and AG26094 to H.S.), the American Diabetes Association (7-07-RA-80 to J.K.K.), and the Ellison Medical Research Foundation (to H.S.). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript. Core resources, including the UMASS Mouse Metabolic Phenotyping

Center, supported by the Diabetes Endocrinology Research Center grant DK32520 were also used. Hayla K. Sluss and Jason K. Kim are members of the UMass DERC (DK32520).

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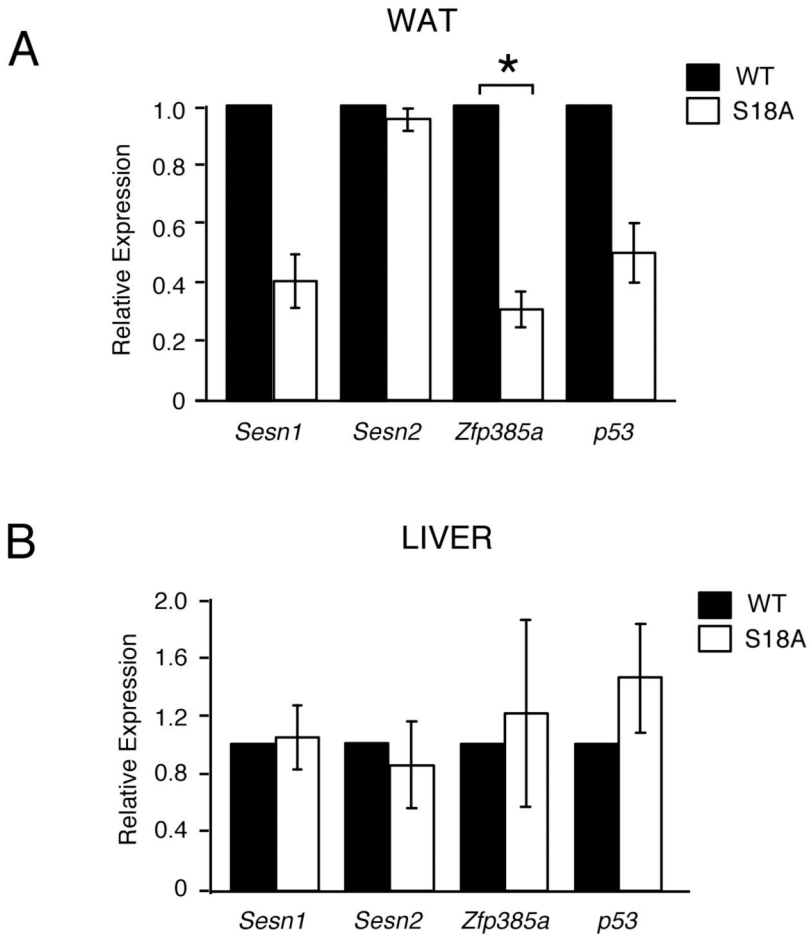

Fig. 1. *Zfp385a* is decreased specifically in WAT of $p53^{S18A(B6)}$ mice

(**A–B**) The expression of *Sesn 1/2*, *Zfp385a*, *p53,* and *Gapdh* mRNA was measured by quantitative real time PCR analysis in 6 month old wildtype mice compared to $p53^{S18A}$ mice in white adipose tissue (WAT) (**A**) and liver (**B**). The amount of *Gapdh* mRNA in each sample was used to calculate relative mRNA expression (mean ± S.E.M.; n = 3, done in triplicate). Statistically significant differences between WT and $p53^{S18A}$ mice are indicated (*, $P < 0.05$).

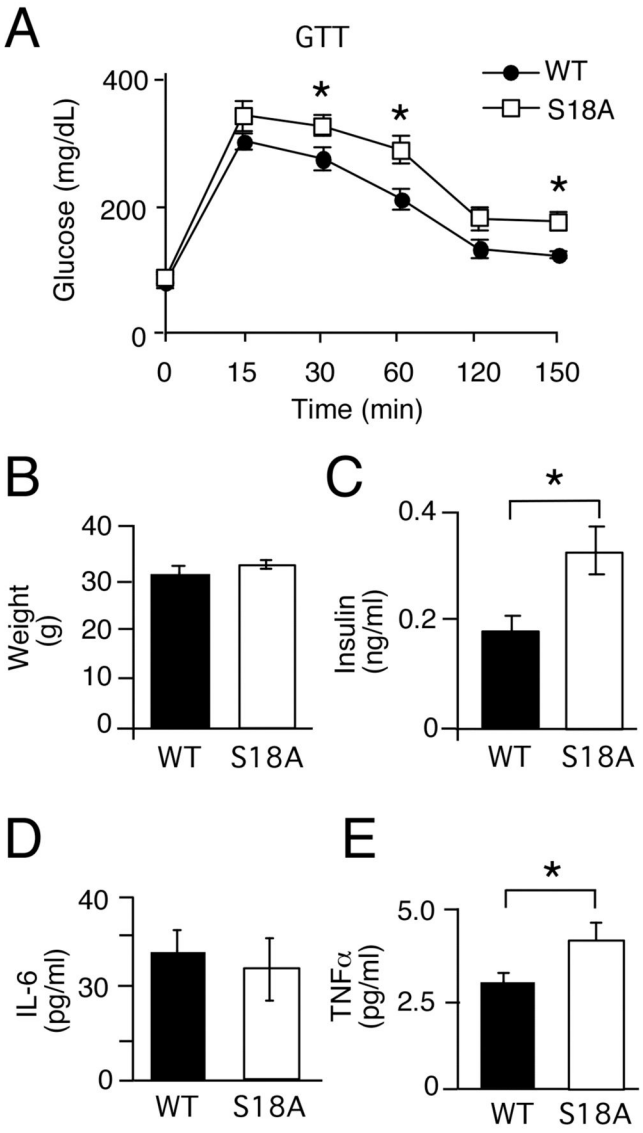

Fig. 2. ***p53*$^{S18A(B6)}$ mice exhibit decreased glucose sensitivity with increased insulin levels**

Wildtype (WT) and *p53*^{S18A} (S18A) mice were maintained on a standard chow diet. Experiments were performed on 6–7 months old animals. (**A**) Glucose tolerance test (GTT). Mice fasted overnight were treated with glucose (1g/kg) by intraperitoneal injection. Blood glucose concentration was measured at the indicated times (mean ± S.E.M.; n = 15). (**B**) Body weight was measured at 6 months (mean ± S.E.M.; n = 15). (**C**) Insulin measurement in mice fasted overnight (mean ± S.E.M.; n = 9). (**D–E**) WT and *p53*^{S18A} mice were fasted overnight and the blood concentration of IL-6 (**D**) and TNFα(E) was measured (mean ± S.E.M.; n = 12). (**A – E**) Statistically significant differences between WT and *p53*^{S18A} mice are indicated (*, $P < 0.05$).

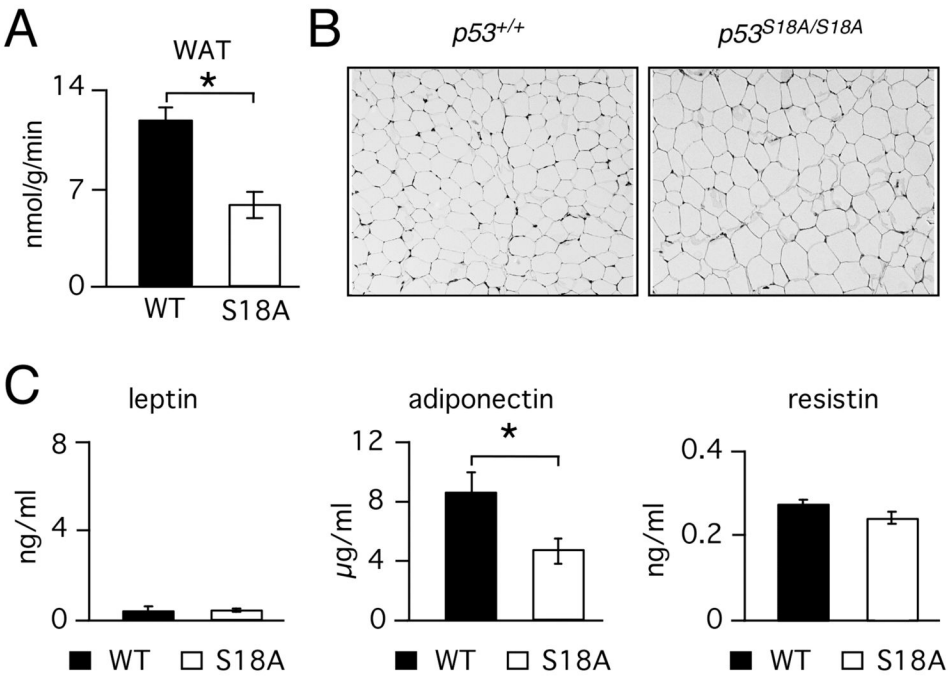

Fig. 3. *p53*$^{S18A(B6)}$ mice mice exhibit adipose tissue insulin resistance and dysfunction

Wildtype (WT) and *p53*^{S18A} (S18A) mice were maintained on a standard chow diet. Experiments were performed on 6–7 month old animals. (**A**) Adipose tissue insulin resistance. Hyperinsulinemia-euglycemic clamp analysis (means ± S.E.M.; n = 5). (**B**) Representative histological sections from epididymal fat pads from WT and *p53*^{S18A} mice. (**C –E**) WT and *p53*^{S18A} mice were fasted overnight and the blood concentration of leptin (**C**), adiponectin (**D**), and resistin (**E**) were measured (mean ± S.E.M.; n = 6). Statistically significant differences are indicated (*, $P < 0.05$).

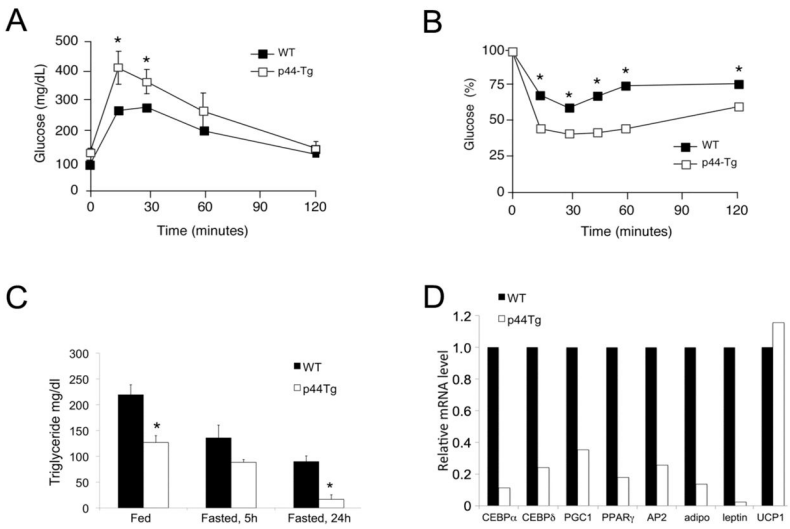

Fig. 4. *p44Tg* mice exhibit decreased glucose sensitivity and defects in adipose tissue

Wildtype (WT) and *p44Tg* mice were maintained on a standard chow diet. Experiments were performed on 3 – 4 month old animals. (**A**) Glucose tolerance test (GTT). Mice fasted overnight were treated with glucose (1g/kg) by intraperitoneal injection. Blood glucose concentration was measured at the indicated times (mean ± S.E.M.; n = 5 – 20). (**B**) Insulin Tolerance Test (ITT). Mice fed ad libitum were treated with insulin (0.75 U/kg) by intraperitoneal injection. Blood glucose was measured at the indicated times (mean ± S.E.M.; n = 5 – 20). Statistically significant differences are indicated (*, $P < 0.05$). (C) Triglyceride levels under fed or fasted conditions for times indicated. (D) Gene expression in WAT from 3 month old male determined by qPCR.

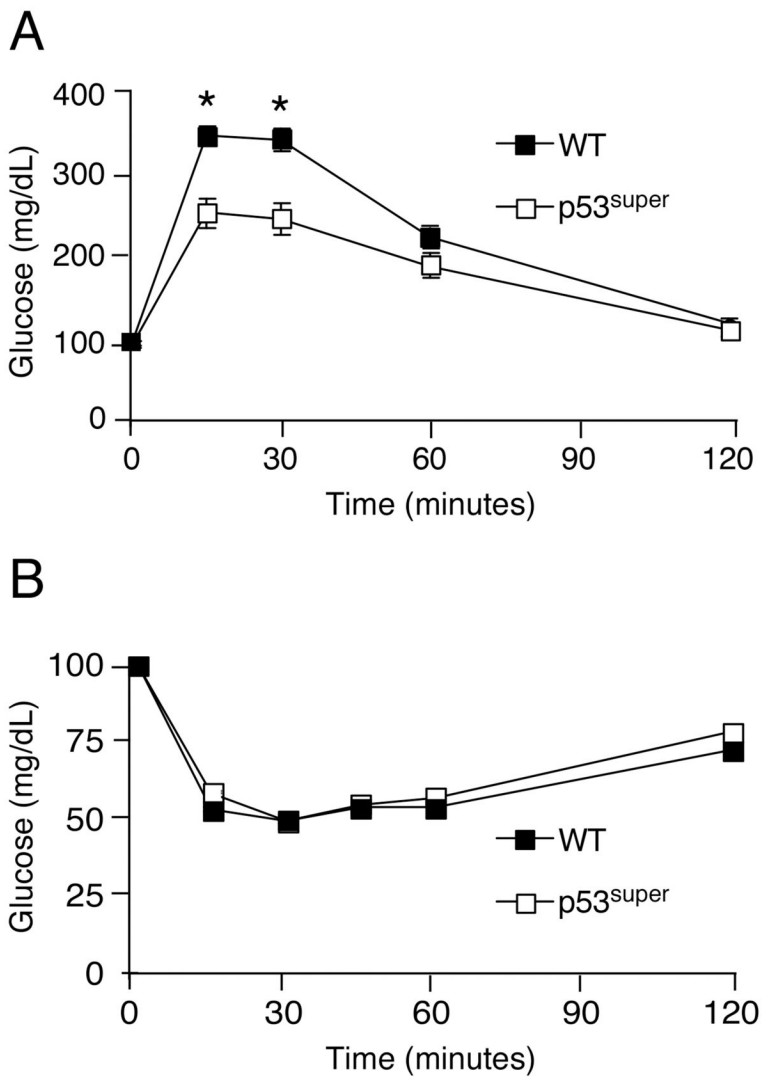

Fig. 5. Super p53 mice exhibit increased glucose sensitivity

Wildtype (WT) and *p53*$^{super/+}$ mice were maintained on a standard chow diet. Experiments were performed on 4 months old animals. (**A**) Glucose tolerance test (GTT). Mice fasted overnight were treated with glucose (1g/kg) by intraperitoneal injection. Blood glucose concentration was measured at the indicated times (mean ± S.E.M.; n = 10). (**B)** Insulin Tolerance Test (ITT). Mice fed ad libitum were treated with insulin (0.75 U/kg) by intraperitoneal injection. Blood glucose was measured at the indicated times (mean ± S.E.M.; n = 10). The mice were fasted overnight and treated without or with insulin (1.5 U/kg body mass) by intraperitoneal injection (30 mins). Statistically significant differences are indicated (*, $P < 0.05$).