p53 regulates the lipid metabolism response to metabolic stress in brown adipose tissue

German Institute of Human Nutrition Potsdam-Rehbruecke

DFG Deutsche Forschungsgemeinschaft German Research Foundation

DZD Deutsches Zentrum für Diabetesforschung

Leibniz Association

Georgia Lenihan-Geels[1], Francisco Garcia-Carrizo[1], Chen Li[2], Moritz Oster[2], Andreas Prokesch[3], Michael Schupp[2], Tim J Schulz[1,4]

[1]**German Institute of Human Nutrition Potsdam-Rehbrücke (DIfE),** Nuthetal, Germany; [2]Institute of Pharmacology, Center for Cardiovascular Research, Charité University Medicine, Berlin, Germany; [3]Institute of Cell Biology, Histology, and Embryology, Medical University Graz, Austria; [4]German Center for Diabetes Research (DZD), München-Neuherberg, Germany

Background

Introduction: Obesity and metabolic dysfunction are highly prevalent worldwide and are associated with increased risk for type 2 diabetes and cardiovascular disease. Brown adipose tissue (BAT) possesses significant potential due its ability for uncoupled respiration and is investigated as a therapeutic target. In addition to its role as a tumour suppressor, p53 is described as a regulator of metabolism in non-transformed cells, and the role of p53 in nutrient flux regulation of adipose tissue is not well understood.

Methods: Acute, BAT-specific p53 knockout (BKO) mice were generated using the tamoxifen-inducible *CreER^{T2}-loxP* system. Control and BKO mice were subjected to fasting or adrenergic activation to investigate the functional role of p53 in directing brown adipocyte nutrient oxidation.

Results: BKO mice demonstrated changed lipid metabolism in BAT and, unexpectedly, also in white fat. Furthermore, the response to acute adrenergic stimulation was blunted in BKO mice, which displayed altered systemic triglyceride metabolism and lipid mobilisation.

Conclusions: Taken together, these data suggest that p53 is an integral part of acute lipid metabolism regulation in BAT. Further investigation will be important in understanding the implication of p53 in nutrient turnover in the context of obesity and metabolic stress.

Knockout model and study design

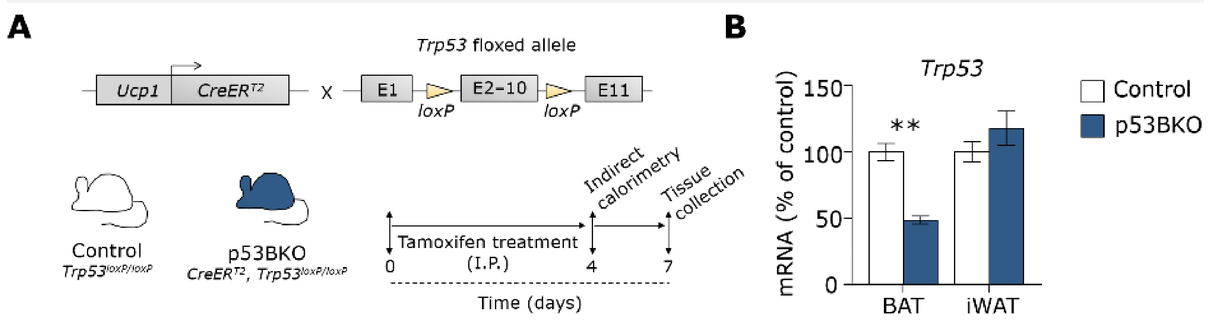

Fig. 1: Acute, brown adipocyte-specific p53 knockout mouse model. (A) Mice carrying the *CreER^{T2}* allele under the control of the *Ucp1* promoter were crossed with *Trp53$^{loxP/loxP}$* mice to generate *Ucp1-CreER^{T2}, Trp53$^{loxP/loxP}$* (p53BKO) mice. Control (mice carrying the *Trp53$^{loxP/loxP}$* but not the *CreER^{T2}* allele) and p53BKO animals were administered with 2 mg of tamoxifen daily for 5 days. **(B)** *Trp53* mRNA expression in BAT and iWAT in control and p53BKO mice. Data expressed as mean + SEM, n=6. Mann-Whitney test. **p<0.01. BAT, brown adipose tissue; iWAT, inguinal white adipose tissue; IP, intraperitoneal.

Metabolic phenotype of p53BKO mice

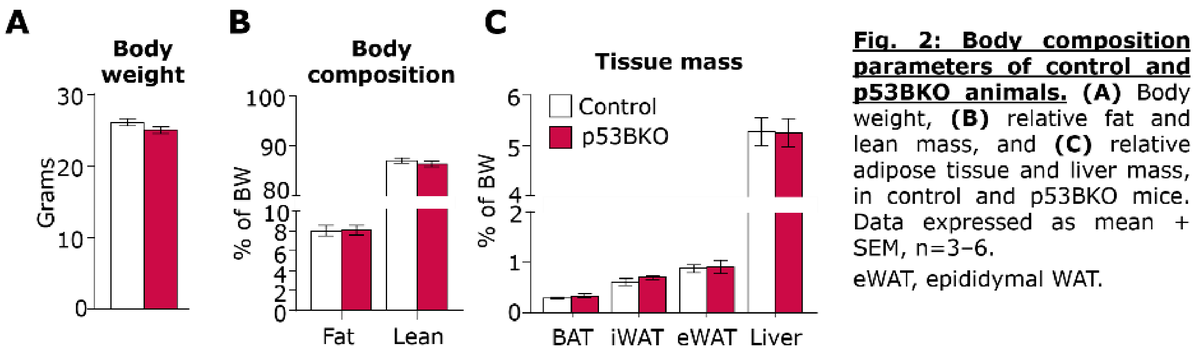

Fig. 2: Body composition parameters of control and p53BKO animals. (A) Body weight, **(B)** relative fat and lean mass, and **(C)** relative adipose tissue and liver mass, in control and p53BKO mice. Data expressed as mean + SEM, n=3–6.
eWAT, epididymal WAT.

Loss of p53 in brown adipocytes disrupts energy metabolism

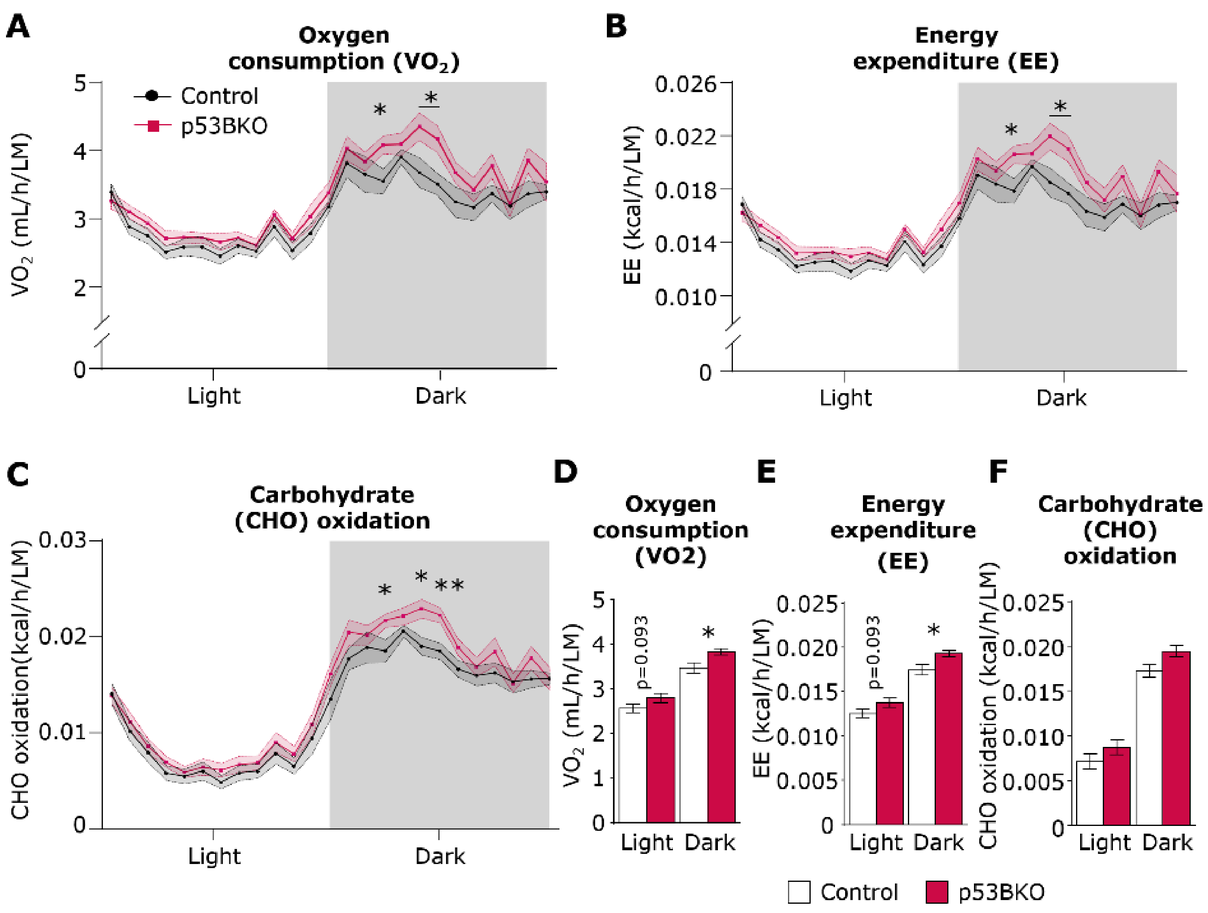

Fig. 3: Energy metabolism parameters. Indirect calorimetry measurements of **(A)** VO_2, **(B)** EE and **(C)** CHO oxidation in control and p53BKO mice. Average **(D)** VO_2, **(E)** EE and **(C)** CHO oxidation. Data expressed as mean + SEM, n=6. Multiple t-tests or Mann-Whitney test. *p<0.05, **p<0.01. CHO, carbohydrate; EE, energy expenditure; LM, lean mass; VO_2, oxygen consumption rate.

Metabolic gene expression profile

Metabolic gene expression profile of brown adipose tissue reveals disrupted glucose and lipid metabolic processes

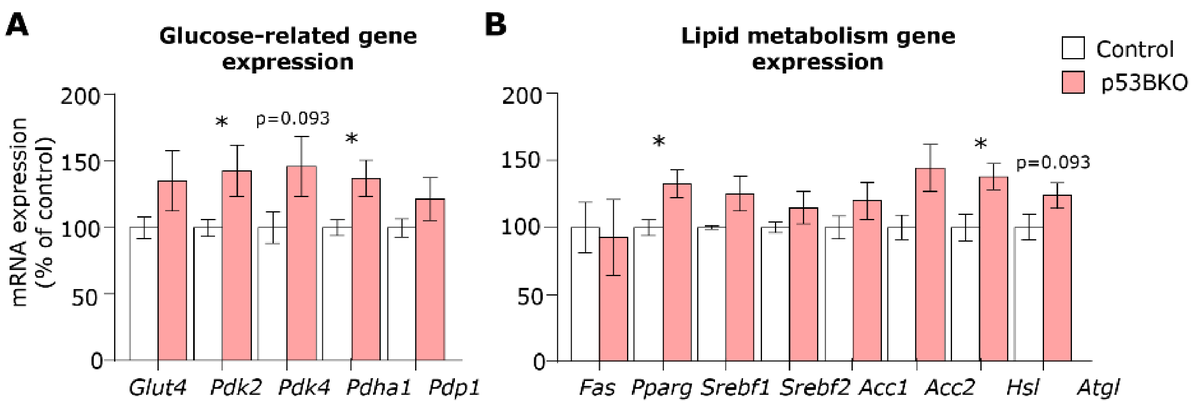

Fig. 4: Altered BAT gene expression profile in p53BKO. Expression of genes regulating **(A)** glucose-related processes and **(B)** lipid metabolic processes in BAT. Data expressed as mean + SEM, n=6. Mann-Whitney test. *p<0.05.

p53 mediates beta-adrenergic response

Administration of beta 3-adrenergic agonist, CL316,243, in mice lacking *Trp53* in brown adipocytes disrupts systemic lipid metabolism

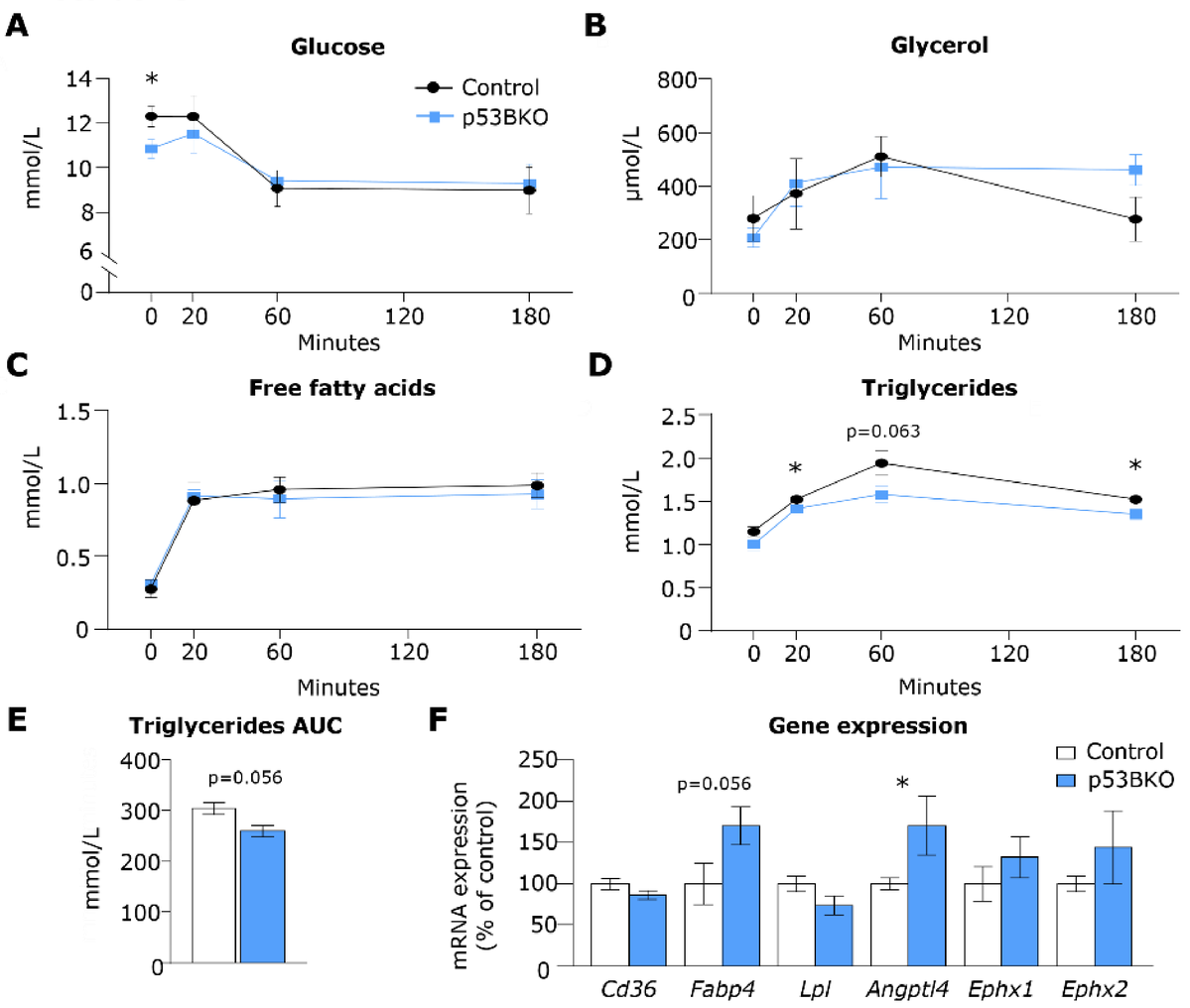

Fig. 5: Lipid metabolism is altered after beta-adrenergic stimulation in p53BKO. Circulating **(A)** glucose, **(B)** glycerol, **(C)** free fatty acids and **(D)** triglycerides at baseline, 20, 60, 120, 180 minutes after CL316,243 injection (IP; 1 µg/g BW). **(E)** Circulating triglyceride levels, calculated as AUC (0–180 min). **(F)** Expression of genes regulating lipid uptake in BAT. Data expressed as mean + SEM. n=5. Multiple t-tests or Mann-Whitney test. AUC, area under the curve.

Acute p53 ablation in brown adipocytes does not influence BAT activation markers following beta-adrenergic stimulation

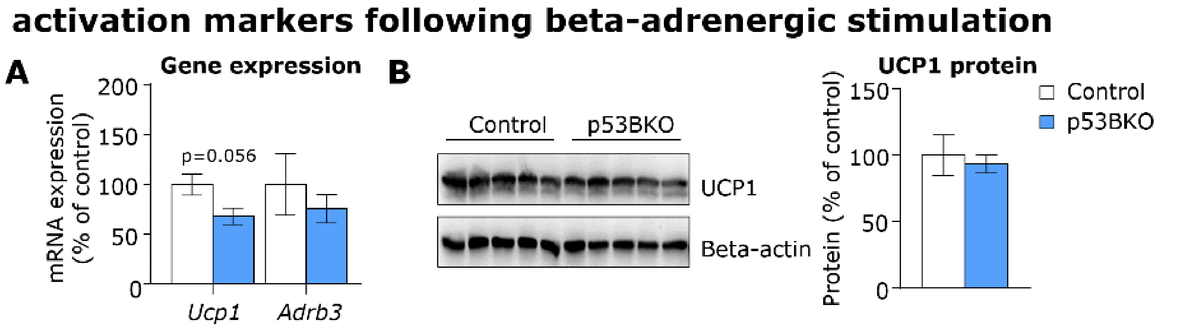

Fig. 6: Markers of beta-adrenergic stimulation. (A) Expression of *Ucp1* and *Adrb3* in BAT. **(B)** UCP1 protein levels in BAT. Data expressed as mean + SEM, n=5. Mann-Whitney test.

Summary

- Acute, brown adipocyte-specific ablation of p53 generates a **hypermetabolic state** by inducing **carbohydrate metabolism** and **energy expenditure**
- Gene expression analysis indicates **alterations in nutrient pathways** following acute loss of p53 in brown adipocytes
- p53 mediates **lipid metabolism** in response to beta-adrenergic stimulation in BAT, possibly via **triglyceride uptake modulation**

Contact: Georgia Lenihan-Geels, phone: +4933200882111, e-mail: georgia.lenihan-geels@dife.de