FINAL REPORT MARIE CURIE ACTIONS - IEF

Project: PHD-Ob-T2D; Researcher: Peter Fraisl

The central aim of the proposed project was to elucidate the potential of prolyl hydroxylases domain (PHD) protein inhibition as a means to interfere with the development of obesity and type 2 diabetes (T2D). To this end we induced obesity via high fat diet (HFD) feeding in PHD-deficient mice and monitored whether PHD deficiency would have consequences on weight gain and obesity-associated medical complications such as insulin resistance (IR) and glucose intolerance (GI), which would ultimately lead to the development of T2D. We also aimed on determination of how loss of PHD function would alter pancreatic β-cell physiology and whether alterations in glucose and lipid homeostasis would reveal a potentially beneficial effect to utilize PHD inhibition as a pharmaceutical intervention to treat obesity and T2D.

Before the onset of this project we have started to extensively characterize the metabolic reprogramming in PHD1-deficient skeletal muscle that protected this tissue of ischemia-induced demise(*1*). We reasoned that the metabolic changes seen in PHD1-deficient skeletal muscle might alleviate the adverse effects observed upon nutritional overload (as in obesity) that consequently lead to disease. In fact, when subjecting lean mice to a glucose tolerance test, we observed a slight but significantly better glucose tolerance in PHD1$^{-/-}$ mice (Fig. 1). No differences were observed in PHD2$^{+/-}$ or PHD3$^{-/-}$ mice (not shown). This finding prompted us to further investigate the underlying mechanism. We found baseline glucose uptake induced in glycolytic muscle and insulin-stimulated glucose uptake induced in highly oxidative muscle such as the heart and diaphragm. In summary PHD1-deficient skeletal muscle takes up more glucose in order to feed enhanced glycolysis that is needed to compensate for the reduction in oxidative phosphorylation. Moreover, we found that the enhanced muscle glycolysis fosters generation and secretion of lactate. This lactate is shunted to the liver to serve gluconeogenesis (GNG) and this glucose returns to the muscle to serve again anaerobic breakdown, completing what is referred to as the Cori Cycle. Consequently we found GNG enhanced in PHD1$^{-/-}$ mice. Under baseline conditions this likely serves to maintain glucose homeostasis preventing harmful hypoglycemia. To find out whether these changes would alleviate obesity-associated risk parameters such as IR and hyperglycemia we subjected these mice to a HFD. To our surprise, PHD1$^{-/-}$ mice gained more weight upon HFD feeding. They also displayed reduced glucose tolerance and hyperinsulinemia indicative of an enhancement of IR (Fig. 2 and 3). Thus, PHD1 inhibition cannot ameliorate the adverse medical consequences of HFD-induced obesity. These findings led to cancellation of aim 3 for PHD1-deficient mice. For further metabolic characterization of PHD1-deficient mice we focused on hepatic alterations as the liver is a central organ in nutritional homeostasis. The liver is also characterized by endogenous variations in oxygen contents, displaying hypoxia in areas surrounding the perivenous vessels exporting blood from the tissue. The impact of such variations in O_2 content on liver function is poorly characterized, but it is presumed that O_2-associated changes in hepatic function impacts on metabolism, detoxification and tumor development. We found that PHD1 regulates several genes that associate with an O_2-dependent gradient in the liver thus causing the liver to alter its metabolism, including enhancement of GNG. We also found clear evidence that hepatic PHD1-deficiency leads to an elevated activation of the canonical NFκB pathway. We are currently investigating the consequences of these changes on tumor development and cancer cell lodging as it occurs during hepatic metastasis development.

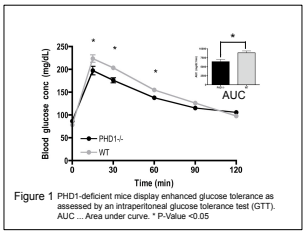

Figure 1 PHD1-deficient mice display enhanced glucose tolerance as assessed by an intraperitoneal glucose tolerance test (GTT). AUC ... Area under curve. * P-Value <0.05

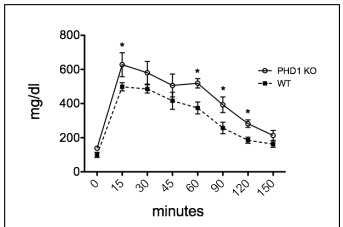

Figure 2 PHD1-deficient mice display impaired glucose tolerance when subjected to HFD. * P-Value <0.05

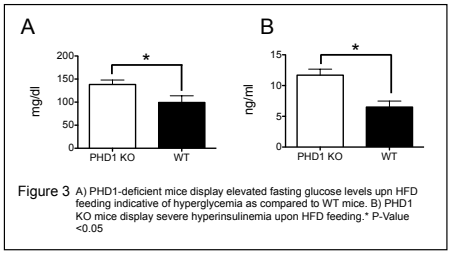

Figure 3 A) PHD1-deficient mice display elevated fasting glucose levels upn HFD feeding indicative of hyperglycemia as compared to WT mice. B) PHD1 KO mice display severe hyperinsulinemia upon HFD feeding.* P-Value <0.05

Moreover, PHD deficiency caused a significant drop in total plasma cholesterol levels, suggesting the PHD1 inhibition could be exploitable for the treatment of hypercholersterolemia.

According to aim 2, we investigated whether PHD1-deficiency would alter β-cell function. We reasoned that the absence of PHD1 might reprogram β-cell metabolism similar to skeletal muscle metabolism and thus impact on glucose-stimulated insulin secretion. We therefore performed a microarray analysis on isolated pancreatic islets. Unfortunately, we did not find changes in gene expression similar to what we found in skeletal muscle. We also did not find changes in insulin secretion upon administration of a glucose bolus *in vivo*. The hyperinsulinemia observed in HFD-mice is most likely a direct consequence of the IR observed in these mice.

No baseline metabolic alterations (e.g. glycemia, insulin levels, GTT, insulin tolerance) could be found in $PHD2^{+/-}$ and $PHD3^{-/-}$ mice (unpublished observations). In order to assess the consequences of PHD2-deficiency on metabolism we therefore decided to pursue the direct approach of generating mice that completely lack PHD2 in specific tissues. To this end we have generated mice that lack PHD2 in the adipose tissue (intercrossing PHD2 floxed mice with a mouse strain that expresses the Cre recombinase in the adipose tissue, *aP2*.Cre). However, these mice were only available after the expiration of the grant period and we are currently investigating how adipose-specific PHD2 deficiency impacts on the development of obesity. Most notably, we have found that PHD2 protein levels are reduced in obese mice as compared to lean counterparts (unpublished observations), indicative that PHD2 may contribute to adipose tissue function during obesity. Similarly we have initiated a liver-specific null of PHD2, but these mice are not available yet due to unforeseen difficulties associated with breeding of these mice. As previously reported, $PHD3^{-/-}$ mice display substantial alterations in the sympathoadrenergic system that consequently leads to significantly diminished levels of adrenalin and systemic hypotension(*2*). Adrenalin can promote glucose deposition and thus impacts on stress induced energetic homeostasis. However, we have not found any baseline alterations of metabolic parameters in PHD3-deficient mice suggesting compensatory mechanisms. In order to circumvent the impact of PHD3-deficiency mediated alterations in the adrenergic system on whole body metabolism and to directly assess the consequence of PHD3 loss on key metabolic tissues we initiated generation of tissue-specific null mice. Again, these mice were not available at the end of the grant period and efforts are under way to characterize changes elicited by tissue-specific ablation of PHD3.

Taken together, we have focused or research efforts on PHD1 deficient animals since these mice displayed already baseline alterations in nutritional homeostasis that indicated a potentially beneficial effect to counteract IR and GI. However, in the HFD-induced obesity model these mice displayed quite the opposite phenotype as what we expected. Addressing whether PHD inhibition of the other two isoforms would reveal a potentially therapeutic benefit was significantly delayed as we decided that the best option is the generation of tissue-specific knock-out mice.

Thus, little can be concluded on the socio-economic impact of the performed research and as whether global or isoform specific PHD inhibition might prove beneficial for the treatment of obesity and T2D. We can, however, conclude that inhibition of PHD1 might aggravate obesity-associated disorders, such as T2D. Consequently, PHD inhibition might not serve as a potential means to treat these diseases, but clearly further studies are needed to provide a distinct answer to this question. Notably, hypoxia, leading to inactive PHDs, is also a hallmark of several diabetic complications, such as vascular dysfunction. Whether the program initiated by PHD inactivation rather acts to alleviate these adverse effects or further promotes them will be of future interest for the treatment of these disorders. In fact, a recent study reported that gene-delivery of HIF-1α (a PHD target) improved re-vascularization in diabetic mice(*3*), indicative of a potentially beneficial effect of PHD inhibition. Direct pharmacologic PHD inhibition, however, has not been assessed and would be of relevant medical importance for future efforts.

1. J. Aragones *et al.*, *Nat Genet* **40**, 170 (Feb, 2008).
2. T. Bishop *et al.*, *Mol Cell Biol* **28**, 3386 (May, 2008).
3. K. Sarkar *et al.*, *Proc Natl Acad Sci U S A* **106**, 18769 (Nov 3, 2009).