Supplementary Background Information

RNA expression changes in white adipose tissue (WAT)

As shown in Figure 1A, strikingly up regulated transcripts in HFD compared to the LFD condition were derived from genes involved in immune response and inflammation such as Rgs1, Atf3, Cd72, and Trem2; and Ubd that probably function as survival factors. One of the most striking down regulated genes in HFD compared to the LFD condition was Cldn22, which plays an important role in tight junction-specific obliteration of the intercellular space. Interestingly, selectively up regulated upon RGZ treatment were two (novel) genes, AI324046 and LOC245281 for which no or very scarce information is available.

Selectively down regulated upon RGZ treatment with the strong PPARγ-activator RGZ in WAT were Igfals, known as the insulin-like growth factor-binding protein complex acid labile subunit, and C6, a complement component, which plays a role in the innate and adaptive immune response, corroborating a recently described link between obesity and the complement system.[1, 2]

Genes, which were up regulated in HFD and down regulated by RGZ treatment were genes like Tph2, tryptophan 5-hydroxylase 2, which may play a still insufficiently unexplored role of protein serotonylation in peripheral insulin resistance.[3]

Even more unexpected genes were the gene Pvalb, known to be involved in relaxation after muscle contraction, Saa3, a major acute phase reactant, and apolipoprotein of the HDL complex. Furthermore, we found S100A8, a calcium- and zinc-binding protein which functions in immune response and inflammatory processes.

Furthermore, we also detected genes that were down regulated in HFD and up regulated by RGZ treatment such as the well-known PPARγ target gene Cidea that is involved in forming

lipid droplets, thereby restricting lipolysis and favoring fat storage; Ptrf, a component of caveolae formation that may support protection of fat cells from lipotoxic effects of elevated fatty acid levels,[4] Cap1, known to be implicated in many developmental and morphological processes; and Slc1a5, an insulin-dependent transporter for L-serine and, to a lesser extent, uptake of L-alanine and L-glutamate. These results may corroborate the known strong insulin sensitizing effects of RGZ treatment.

RNA expression changes in liver

As shown in Figure 1B, strikingly up regulated genes in HFD compared to the LFD condition were genes involved in fat metabolism such as apolipoprotein of the HDL complex and genes involved in immune processes and apoptosis such as Saa1 and the gene of the iron-trafficking protein Lcn2.

The most striking down regulated genes in HFD compared to the LFD condition were Fbxo21, a substrate-recognition component of the SCF-type E3 ubiquitin ligase complex, Spon2 that functions in cell adhesion, and Cyp17a1, known for conversion of pregnenolone and progesterone to dehydroepiandrosterone (DHEA) and androstenedione, suggesting potential hormonal dysregulation by HFD.

Genes, which were instead down regulated in HFD and up regulated after RGZ treatment were various immune and inflammatory response genes such as Ccl5, a chemoattractant for immune cells, the cytokines Cxcl1 and Cxcl9, and Saa3, a major acute phase reactant.

Genes, which were up regulated in HFD as well as by RGZ treatment were the well-known PPARγ target gene Cidea (similar in WAT!), Serpina4-ps1 (a proteinase inhibitor) and Cfd for which no or very scarce information was available.

These data indicated adipose and liver tissue-specific gene regulation processes but also showed some overlapping effects of RGZ treatment due to specific activation of PPARγ and its target genes such as Cidea.

Protein expression changes in liver

As displayed in Figure 2B, also the entire set of identified proteins of the liver showed more varying regulations compared to the RNA expression data set.

A closer look revealed that the following functionally diverse proteins were up regulated exclusively in HFD state: PSAP, a protein of the sphingolipid metabolic process; BAT2 that may be involved in pre-mRNA splicing; and CAST that specifically inhibits calpains (the physiological role of the cysteine proteases is still poorly understood). In contrast, only down regulated proteins in the HFD compared to the LFD state were two proteins involved in vitamin metabolism (folic acid, B6), namely DHFR, a key enzyme in folate metabolism and PDXDC1. This is in concordance to drastically down regulated vitamins in HFD (discussed in metabolomics section). Furthermore, several ribosomal proteins, like RPS5, RPL18, RPL6, RPLP2, RPL22 were down regulated. As in WAT, ACTA2, known in the context of muscle physiology was down regulated in liver.

Specifically up regulated proteins upon RGZ treatment were SYNE1 that is involved in maintaining subcellular spatial organization; and one of the prime targets of PPARγ, the lipid transport protein FABP4.

Proteins, which are up regulated in HFD and counteractively down regulated by RGZ treatment were proteins with various functions including ZC3H18, a zinc finger CCCH domain-containing protein; CDKN2AIP that activates p53; NUCKS1, a nuclear ubiquitous casein and

cyclin-dependent kinase substrate; PDLIM5, a component of the protein complex eIF4F, which is involved in the recruitment of mRNA to the ribosome; and RRBP1, a ribosome receptor that mediates interaction between the ribosome and the endoplasmic reticulum membrane.

Proteins, which are down regulated in HFD and counteractively up regulated by RGZ treatment featured diverse functions such as COPZ1 that associates with Golgi non-clathrin-coated vesicles; ANK3, a membrane-cytoskeleton linker for cell adhesion molecules; LOH11CR2A that may play a role in tumorigenesis as a tumor suppressor; and STOML2 that is involved in biogenesis and activity of mitochondria, indicating metabolic effects of RGZ-based activation of PPARγ. PSME2 (proteasome activator complex subunit 2) seems to be the most interesting of them, known to be implicated in immunoproteasome assembly and required for efficient antigen processing by enhancing the generation of class I binding peptides through altered cleavage pattern of the proteasome. This is supported by recent findings, in which hyperglycemia and diabetes were found to modulate cellular proteasome function by increased levels of PMSE2,[5] as well as that PSME2 and the immunoproteasome are necessary for adaptation to oxidative stress.[6]

Metabolite changes in white adipose tissue

Allantoin, a product of nonenzymatic urate oxidation and marker of increased oxidative stress, was recently found to be increased type 1 diabetic patients (Lanza, Zhang et al. 2010). Concordantly, in our study allantoin was 2-fold elevated in HFD, upon RGZ treatment it was even 10-fold increased.

Betaine insufficiency is associated with the metabolic syndrome, lipid disorders and diabetes and has therefore been used in treatments of liver disorders. In WAT, betaine levels are unchanged in HFD versus LFD state, but decreased 3-fold upon RGZ treatment.

Metabolite changes in liver

Sarcosine is known to be increased in invasive prostate cancer and is claimed to be a specific biomarker for this cancer. Sarcosine levels were unchanged between LFD and HFD states, but increased 10-fold upon RGZ treatment, suggesting potentially adverse effects of RGZ drug treatment in liver.

Further, acetoacetate can be produced in the human liver under certain conditions of poor metabolism leading to excessive fatty acid breakdown (diabetes mellitus leading to diabetic ketoacidosis). Concordantly, HFD-feeding was associated with a 1.6-fold increase in liver acetoacetate, whereas treatment with the antidiabetic RGZ further reduced the liver acetoacetate levels by a factor of 6.

References

(1) Oberbach, A.; Bluher, M.; Wirth, H.; Till, H.; Kovacs, P.; Kullnick, Y.; Schlichting, N.; Tomm, J. M.; Rolle-Kampczyk, U.; Murugaiyan, J.; Binder, H.; Dietrich, A.; von Bergen, M. Combined proteomic and metabolomic profiling of serum reveals association of the complement system with obesity and identifies novel markers of body fat mass changes. *J. Proteome Res.* **2011,** 10 (10), 4769-4788.

(2) Satoskar, A. A.; Shapiro, J. P.; Bott, C. N.; Song, H.; Nadasdy, G. M.; Brodsky, S. V.; Hebert, L. A.; Birmingham, D. J.; Nadasdy, T.; Freitas, M. A.; Rovin, B. H. Characterization of

glomerular diseases using proteomic analysis of laser capture microdissected glomeruli. *Mod. Pathol.* **2012,** 25 (5), 709-721.

(3) Walther, D. J.; Peter, J. U.; Bashammakh, S.; Hortnagl, H.; Voits, M.; Fink, H.; Bader, M. Synthesis of serotonin by a second tryptophan hydroxylase isoform. *Science* **2003,** 299 (5603), 76.

(4) Meshulam, T.; Breen, M. R.; Liu, L.; Parton, R. G.; Pilch, P. F., Caveolins/caveolae protect adipocytes from fatty acid-mediated lipotoxicity. *J. Lipid Res.* **2011,** 52 (8), 1526-1532.

(5) Aghdam, S. Y.; Gurel, Z.; Ghaffarieh, A.; Sorenson, C. M.; Sheibani, N. High glucose and diabetes modulate cellular proteasome function: Implications in the pathogenesis of diabetes complications. *Biochem. Biophys. Res. Commun.* **2013,** 432 (2), 339-344.

(6) Pickering, A. M.; Koop, A. L.; Teoh, C. Y.; Ermak, G.; Grune, T.; Davies, K. J. The immunoproteasome, the 20S proteasome and the PA28alphabeta proteasome regulator are oxidative-stress-adaptive proteolytic complexes. *Biochem. J.* **2010,** 432 (3), 585-594.