Supplementary discussion:

1. Variation of acetylated peptide levels in biological replicates

While biological replicates of pooled samples were not measured in the present experiments, in ongoing acetlyome studies with an unrelated mouse model (the lipin 1 deficient, fatty liver dystrophy (fld) mouse), we have performed biological replicates (n=3) on pooled mouse skeletal muscle (quadriceps) samples (n'=3). Comparisons of the three pooled samples show that the standard deviation of acetylated peptides varies from about 10% to about 70% (**Supplementary Table 3**). While these results do not directly address variance in the present data, they do suggest that variance among pools of 5 animals should be considerably less than the 3-fold cutoff used in the present approach.

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2. Acetylome differences under fasted/re-fed transition in different organs

Brown adipose has a mixture of hyper- and hypo-acetylated proteins in the re-fed *vs*. fasted states, which shows some intriguing targets, which may indicate brown adipose has a metabolic role with re-feeding. Aconitate hydratase (ACO2), trifunctional protein subunit α (TP-α), propionyl CoA carboxylase alpha chain (PCCA-α) are hypoacetylated, while hydroxymethylglutaryl-CoA lyase (HMGCL) and cytochrome b5 type B (Cyt5b) and glycine cleavage system H protein (GCSH) are hyperacetylated. Presumably, ACO2 and PCCA-α hypoacetylation increases TCA cycle flux, while TP-α hypoacetylation decreases β-oxidation, which would be a similar effect to EHHADH[9], and to that hypothesized for ECH1 with respect to liver β-oxidation. HMGCL is needed for ketogenesis, but in brown adipose, hyperacetylation probably increases activity and provides increased flux into the TCA cycle, as it catalyzes final

step in leucine degradation[36], and amino acids are quickly disposed of after re-feeding. GCSH is part of the glycine cleavage system, which is responsible for a substantial proportion of whole body glycine flux and constitutes a major route for the generation of 1-carbon units as 5,10-methylenetetrahydrofolate (methyleneTHF). Hyperacetylation of GCSH probably increases its activity, as glycine would need increased disposal with re-feeding. White adipose, in contrast, just shows hypoacetylation of VDAC1 and ATP coupling factor 6.

The kidney shows dramatically increased acetylation in re-fed/fasted protein targets. F_oF_1-ATP synthase, HSP-60, HSP-70/GRP-75, HMGCL, α-KGDH, ACAT1 all have increased acetylation in the re-fed versus fasted states. After re-feeding there would be additional demands on the kidney to aid in the disposal of metabolites, as well as for the kidney itself to best use available nutrients for its own needs. For the kidney, glucose oxidation, while not the major source of renal energy, is crucial in sodium, potassium, and phosphate reabsorption[37], and fasted/fed protein acetylation changes in the kidney may help to adjust the energy status to regulate fluctuations in renal electrolyte loads. As stated for brown adipose, hyperacetylation of HMGCL probably increases its activity. ACAT1 is involved in fatty acid oxidation, and hyperacetylation probably increases its activity for use as an energy source, tying in with the hyperacetylation of F_oF_1-ATP synthase to maximize energy production. Hyperacetylation of α-KGDH, in this context, probably increases α-ketoglutarate dehydrogenase activity, matching increased TCA flux to energy generation.

The role for the HSP-60, HSP-70/GRP-75 (otherwise known as mortalin), chaperones may be to maintain mitochondrial function in one of these states in a tissue specific fashion. For example,

100-fold increases in re-fed/fasted acetylation ratios were seen for Hsp-60 and mortalin in the kidney. Mortalin and Hsp 60 heat shock protein are important for the maintenance of mitochondrial function. In yeast, decreases of Hsp60 cause iron increase and negatively affect the activity of Fe/S dependent enzymes (such as those seen in the TCA cycle) upon oxidative stress[38]. Mortalin is essential for the import and maturation of the yeast frataxin homolog (Yhf1), and for Fe/S cluster assembly in mitochondria[39]. Frataxin is thought to support the biogenesis of Fe/S clusters, such as those that include aconitase and succinic dehydrogenase, and respiratory chain complexes[39, 40]. There are 12 different Fe-S clusters which shuttle electrons through complexes I-III[41]. Acetylation may be part of this chaperone regulation mechanism. Evidence for acetylation of another chaperone, Hsp90, indicates its acetylation status may propagate complex allosteric information throughout the chaperone.[41] Acetylation of K294 of Hsp 90, or mutations to mimic acetylation, prevented stable complex formation with its chaperone targets, and prevented Hsp90's ability to activate purified Chk1 kinase[42]. Hyperacetylation may impair Hsp70 as well as Hsp90 function, and Hsp70 function has been shown to be important for maintaining Hsp90 chaperone function.[43] Chaperones have been reported to work collaboratively with the histone deacetylases (HDACs, reviewed in 31). Deacetylated Hsp70, catalyzed by HDAC10 or by another deacetylase, joins the HSP90/chaperone client complexes to help with correct folding of the clients. Acetylation of Hsp70, might cause incorrect folding of proteins or facilitate the subsequent degradation of misfolded proteins.[43] The question then becomes, what is the relevance of this for fasted/fed regulation of mitochondrial function and the respiratory chain for the kidney? The Hsp90 amd Hsp70 studies were done in K562 erythroleukamia type cells[43], and COS7, SkBr3, MCF7, and NIH 3T3 cells[42]. Different co-chaperone complexes may form in normal cells, as opposed to tumor cell lines. Future work will

characterize the role of acetylation in fasted/fed regulation of kidney metabolic function, as it is premature to extrapolate that increased fed *vs.* fasted acetylation of the chaperones seen in kidney leads to their deactivation in the fed state.

The brain mainly shows an increase in acetylation of electron transfer flavoprotein subunit β, which may reflect increased energy generation in the fed *vs.* fasted state. Acetylation seen mainly in either the fasted or re-fed states may function to maintain metabolic flux or function under those conditions, and **Figure 6** denotes these targets also.

A consideration to be treated in future work is what regulates tissue specific acetylation? One might hypothesize that the increases in insulin seen in the re-fed *vs*. fasted state may be driving the acetylation changes. However, there is no acetylation pattern one can recognize for insulin sensitive tissues, such as heart muscle, skeletal muscle, adipose tissue and liver *vs*. tissues whose metabolism is not directly regulated by insulin, such as brain and kidney, as discussed previously. It may be that there is a strong feedback component of metabolic regulation that drives acetylation, and this self-regulation may be another layer of control to metabolism during the fasted/re-fed transition in the kidney, as well as for insulin sensitive tissues, such as the liver. Clearly, the increase in MDH2 acetylation in response to glucose, and the increase in EHHADH acetylation in response to fatty acids[9], supports the idea of self-regulation of metabolism, via acetylation that may be dependent on the acetyl CoA response to these fuels. For insulin sensitive tissues, the increase in glucose transport or glucose metabolism may accentuate this self-regulation response. Insulin action has synergistic pathways for metabolism regulation, such as serine/threonine and tyrosine phosphorylation, and metabolite feedback can act through the

hexosamine biosynthetic pathway, and AMPK signaling, as well as acetylation. *In vivo*, insulin can modulate inter-organ fuel exchange that can affect the acetylation response, such as, to decrease adipose lipolysis and affect the flow of fatty acids into tissues, or to decrease hepatic and kidney glucose production. Kidney and liver are in direct contrast for the pattern of acetylation in the re-fed to fasted transition, with kidney showing hyperacetylation and liver showing hypoacetylation and future studies with examine the significance of these different patterns, for which metabolomics will play a defining role.

Supplementary Table 3. Study of acetylated peptide level variations in biological replicate samples using fasted and re-fed FLD knockout mouse liver samples.

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K.FDDPK@FEVIDKPQS.-			
sample	**area**	**sample**	**area**
fasted 1	1.08E+09	**re-fed 1**	1.30E+09
fasted 2	1.36E+09	**re-fed 2**	1.06E+09
fasted 3	1.30E+09	**re-fed 3**	1.07E+09
	standard deviation/average		**standard deviation/average**
fasted	11.8%	**re-fed**	11.9%

R.LATALQK@LEEAEK.A			
sample	**area**	**sample**	**area**
fasted 1	7.77E+08	**re-fed 1**	1.28E+09
fasted 2	1.46E+09	**re-fed 2**	1.01E+09
fasted 3	1.22E+09	**re-fed 3**	6.67E+08
	standard deviation/average		**standard deviation/average**
fasted	30.1%	**re-fed**	31.2%

K.LKGTEDEVEK@YSESVK.D			
sample	**area**	**sample**	**area**
fasted 1	4.05E+07	**re-fed 1**	5.32E+07
fasted 2	5.26E+07	**re-fed 2**	2.44E+07
fasted 3	3.73E+07	**re-fed 3**	2.22E+07
	standard deviation/average		**standard deviation/average**
fasted	18.6%	**re-fed**	52.0%

K.VLDPEGK@GTIKK.Q			
sample	**area**	**sample**	**area**
fasted 1	1.57E+06	**re-fed 1**	1.35E+06
fasted 2	3.06E+06	**re-fed 2**	2.52E+06
fasted 3	1.56E+06	**re-fed 3**	2.84E+06
	standard deviation/average		**standard deviation/average**
fasted	41.8%	**re-fed**	35.1%

R.AQK@DEEKMEIQEIQLK.E			
sample	**area**	**sample**	**area**
fasted 1	1.34E+08	**re-fed 1**	1.96E+08
fasted 2	2.71E+08	**re-fed 2**	5.30E+07
fasted 3	not observed	**re-fed 3**	1.16E+08
	standard deviation/average		**standard deviation/average**
fasted	47.8%	**re-fed**	58.9%

K.IPVPEDK@YTALVDQEEKEDVK.S

sample	area	sample	area
fasted 1	1.52E+07	**re-fed 1**	2.17E+07
fasted 2	1.79E+07	**re-fed 2**	1.70E+07
fasted 3	2.18E+07	**re-fed 3**	3.01E+07
	standard deviation/average		**standard deviation/average**
fasted	18.1%	**re-fed**	28.9%

K.SLEAQAEK@YSQK.E

sample	area	sample	area
fasted 1	3.88E+06	**re-fed 1**	4.84E+06
fasted 2	4.78E+06	**re-fed 2**	1.58E+06
fasted 3	2.87E+06	**re-fed 3**	1.79E+06
	standard deviation/average		**standard deviation/average**
fasted	24.5%	**re-fed**	66.7%