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REVIEW ARTICLE

Mitochondrial protein acetylation as a cell-intrinsic, evolutionary driver of fat storage: Chemical and metabolic logic of acetyl-lysine modifications

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Abstract

Hormone systems evolved over 500 million years of animal natural history to motivate feeding behavior and convert excess calories to fat. These systems produced vertebrates, including humans, who are famine-resistant but sensitive to obesity in environments of persistent overnutrition. We looked for cell-intrinsic metabolic features, which might have been subject to an evolutionary drive favoring lipogenesis. Mitochondrial protein acetylation appears to be such a system. Because mitochondrial acetyl-coA is the central mediator of fuel oxidation and is saturable, this metabolite is postulated to be the fundamental indicator of energy excess, which imprints a memory of nutritional imbalances by covalent modification. Fungal and invertebrate mitochondria have highly acetylated mitochondrial proteomes without an apparent mitochondrially targeted protein lysine acetyltransferase. Thus, mitochondrial acetylation is hypothesized to have evolved as a nonenzymatic phenomenon. Because the pK_a of a nonperturbed Lys is 10.4 and linkage of a carbonyl carbon to an ε amino group cannot be formed with a protonated Lys, we hypothesize that acetylation occurs on residues with depressed pK_a values, accounting for the propensity of acetylation to hit active sites and suggesting that regulatory Lys residues may have been under selective pressure to avoid or attract acetylation throughout animal evolution. In addition, a shortage of mitochondrial oxaloacetate under ketotic conditions can explain why macronutrient insufficiency also produces mitochondrial hyperacetylation. Reduced mitochondrial activity during times of overnutrition and undernutrition would improve fitness by virtue of resource conservation. Micronutrient insufficiency is predicted to exacerbate mitochondrial hyperacetylation. Nicotinamide riboside and Sirt3 activity are predicted to relieve mitochondrial inhibition.

Keywords

Ac-coA, calorie restriction, lipogenesis, NAD^+, overnutrition, oxaloacetate, pK_a, Sirt3

History

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Animal evolution

Animals, by definition, are heterotrophic organisms that acquire macronutrients from other organisms. Because animals cannot be at the bottom of the food chain, competition creates a reward system for acquiring, conserving and retaining resources. Animals evolved in parallel in many different environments, such that modern animals are remarkably numerous and diverse. Although no single beetle, insect, mollusk or vertebrate ended up with hegemonic control of planetary macromolecules, many of the systems for resource acquisition, conservation and retention are conserved.

The ability to survive, migrate and take advantage of newly discovered riches is fundamental to animal success. Indeed, the ability to acquire and digest complex macromolecular structures made particular organisms such as termites remarkably successful. Because resource availability is never guaranteed into the future and can be expected to be limited in lengthy competitions, the ability to survive famine is among the most highly selected traits over 500 million years of animal evolution.

Animal metabolic pathways have remarkable efficiencies: cellular and extracellular material from other organisms is broken down into glycolytic and lipolytic inputs. Protein is digested to amino acids and the corresponding keto acids. That which is needed to make sugars, building blocks and macromolecules is directed into anapleurotic and biosynthetic processes. That which can be oxidized in the citric acid cycle is consumed to generate ATP.

In animals, all three classes of macronutrients have a storage form that protects against future needs. Carbohydrates are stored as glycogen, which can be phosphorylyzed to supply glucose-1-phosphate on demand. Protein anabolism in muscle can be reversed by proteolysis and conversion to other metabolites, such as in the glucose-alanine cycle. In addition, dietary fat can be stored and excess fuel is converted to fat. Of the three storage macromolecules, fat is the highest in energy content and the only one that does not require hydration to store.

Regulatory mechanisms of metabolism have been gleaned from many systems including bacteria, yeast and vertebrates.

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However, hormonal regulation of metabolism and energy balance is best understood in vertebrates. Complex hormonal regulatory systems control multiple levels of energy conservation from the signaling pathways that regulate anabolism and catabolism within tissues to organismal feeding behaviors and satiety. For example, the mobilization of glucose-1-phosphate from glycogen is stimulated by the hormone, glucagon, which is released from pancreatic α-cells in response to decreasing blood glucose. Glucagon binds to G-protein-coupled receptors in liver and other tissues, producing successive activation of cAMP-dependent protein kinase, phosphorylase kinase and glycogen phosphorylase to release glucose-1-phosphate from glycogen (Jiang & Zhang, 2003). The glucose-alanine cycle and the processes of lipogenesis and lipolysis are also controlled by ancient, hormonal systems that regulate whole body energy balance (Felig, 1973; Scherer *et al*., 2011). In addition, in recent years, hormones produced by fat, the gut and the brain, and which act in multiple tissues, have been found to control hunger, feeding behavior, the set point for fat storage, and other mechanisms that control the disposition of fat (Meier & Gressner, 2004).

In addition to hormonal systems for carbon storage and energy metabolism, we considered whether a more ancient, cell-intrinsic mechanism might underlie lipogenesis. Here, we hypothesize that mitochondrial protein acetylation and acylation is an ancient, conserved system that leads to inhibition of fuel utilization when energy is in excess, producing a cell-intrinsic switch to storage of fat.

Our model proposes that mitochondrial protein acetylation creates an erasable, covalent memory of an overfed state, whose initial metabolic signature consists of high acetyl-coA (Ac-coA), high NADH and high ATP. This model does not require a highly evolved mitochondrial protein Lys acetyltransferase to modify fuel-utilizing enzymes. Instead, we propose that organisms evolved in a manner that put critical Lys residues of mitochondrial enzymes ''in harm's way'' by depressing the pK_a values of acetylation target sites, thereby rendering them susceptible to chemical modification – typically inhibition – during times of overnutrition. This would reduce the ability of mitochondria to restore rapid fuel-utilization after periods of plenty, and tend to promote the storage of fat.

This model simultaneously explains the ability of animals to survive scarcity, the tendency of modern humans to gain weight and experience mitochondrial decline in environments of continual energy excess, and – because of the micronutrient requirements for protein acetylation and deacetylation – explains why macronutrient overnutrition with a scarcity of micronutrients promotes lipogenesis.

Fuel utilization in mitochondria

For the citric acid cycle to oxidize fuel, there must be fuel entering mitochondria as pyruvate, fatty acids and/or amino acids. In addition, mitochondria possess a saturable pool of coenzyme A (coA) and a pool of hydride transfer cofactors that must be in the oxidized, that is NAD^+ and FAD, states for the citric acid cycle to run. After formation of Ac-coA or other anapleurotic inputs, the citric acid cycle requires free coA for the α-ketoglutarate dehydrogenase (α-KGDH) complex to form succinyl-coA. In addition, there must be GDP or ADP for the succinyl-coA synthetase reaction to form succinate, and there must be a supply of each citric acid cycle intermediate to keep the cycle running. Fuel oxidation is coupled to function of the electron transport chain (ETC) in which O_2 must be present as the ultimate electron acceptor – this allows reoxidation of NADH and $FADH_2$ back to NAD^+ and FAD (Figure 1).

The oxidizing state of hydride transfer coenzymes is critical for the direction of multiple steps in the citric acid cycle, especially formation of oxaloacetate from malate by malate dehydrogenase 2 (MDH2). Under standard reaction conditions, that is, 1 M NAD^+, 1 M malate, 1 M NADH and 1 M oxaloacetate, the fuel oxidizing direction of MDH2 ($\text{malate} + NAD^+ \rightarrow \text{oxaloacetate} + \text{NADH}$) is thermodynamically unfavorable by ~30 kJ/mol. The thermodynamic favorability of citrate synthase the ''first'' step in the citric acid cycle, drives the cycle forward so long as there is NAD^+ to produce malate and fuel to produce Ac-coA. Reoxidation of NADH to NAD^+ and continuous production of malate allows oxaloacetate, the limiting substrate, to be formed. Although reversal of the MDH2 reaction is highly favorable, the collision of oxaloacetate with citrate synthase and Ac-coA allows oxaloacetate to be converted to citrate.

These characteristics create several speed limits that collectively govern the citric acid cycle. First, if excessive fuel enters mitochondria, the mitochondrial coA pool could be largely acetylated/acylated and have little free coA available for α-KGDH to function. Second, when the ETC is running at capacity, the hydride transfer coenzymes will begin to accumulate in reduced (NADH and $FADH_2$) forms, which limit formation of the oxaloacetate that is necessary for Ac-coA to enter the cycle at citrate. By definition, if the ETC is running at capacity and protons are being pumped into the mitochondrial intermembrane space for maximal ATP generation, the intermembrane space is maximally acidified. This also means that the pH in the mitochondrial matrix will be alkalinized, potentially to a value as high as 8.2 (Santo-Domingo & Demaurex, 2012).

Consider the consequences of high mitochondrial Ac-coA, ATP and NADH, which may occur in a state of overnutrition in the liver. In addition to MDH2 running backwards at high NADH, pyruvate dehydrogenase (PDH) and citrate synthase are negatively regulated by NADH, and multiple enzymes are inhibited by ATP. In the early steps of the citric acid cycle, retardation or reversal (Des Rosiers *et al*., 1994) of isocitrate dehydrogenase (IDH) and inhibition of α-KGDH result in isocitrate flowing back to citrate. In the latter steps of the cycle, malate accumulates, largely due to the fact that the equilibrium for the MDH2 reaction is strongly in favor of NAD^+ and malate. Citrate and malate are the predominant organic acids exported by mitochondria into cytosol during energy excess. These signals of mitochondrial oversufficiency are important because malate export from liver mitochondria supplies gluconeogenesis, which is diabetogenic and indirectly contributes to lipogenesis. Cytosolic citrate is the direct precursor of cytosolic Ac-coA, which is used for production of malonyl-coA and lipogenesis.

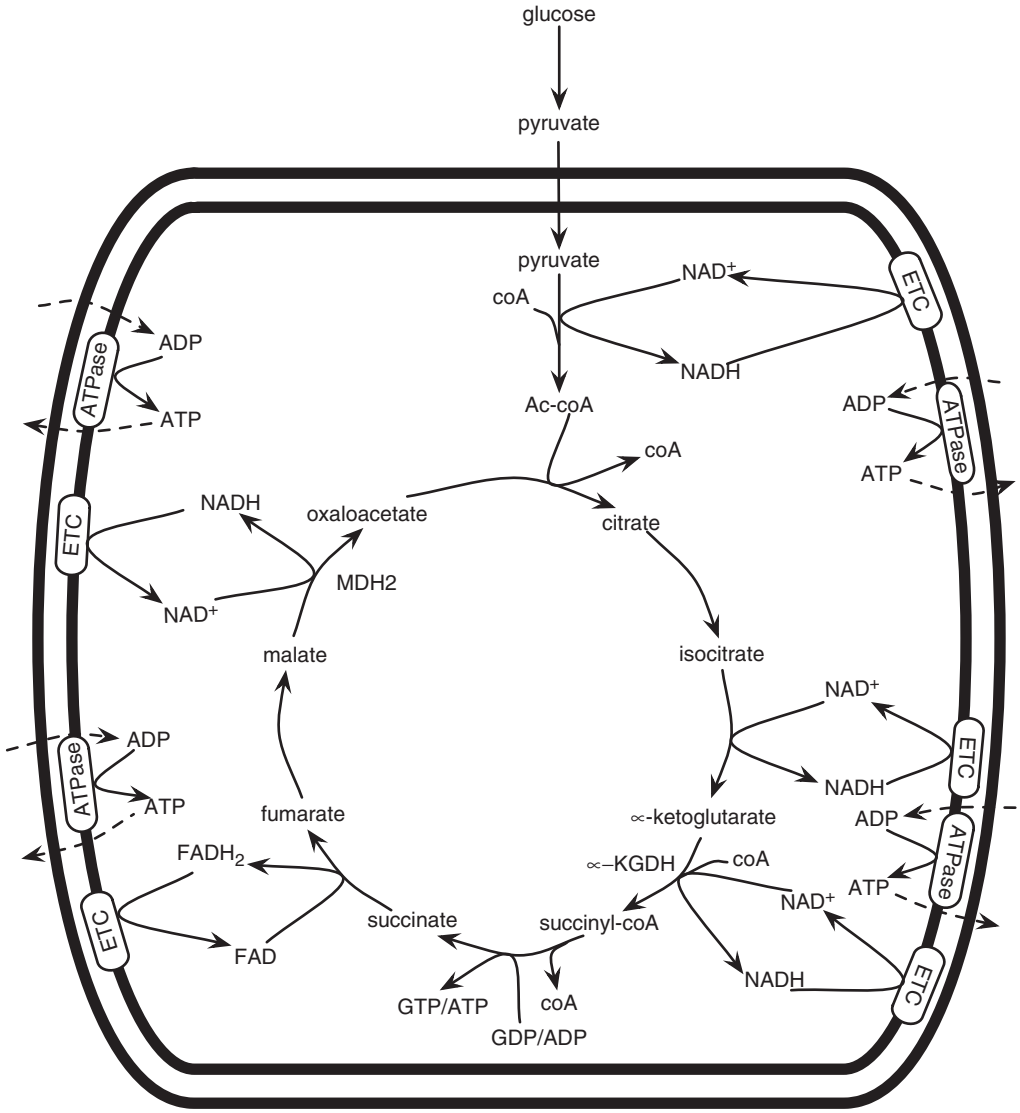

Figure 1. Fuel oxidation by mitochondria. Complete oxidation of pyruvate is depicted. Progression through the citric acid cycle depends on free coA, ADP and the reoxidation of NADH and $FADH_2$ by the electron transport chain (ETC). The net result is fuel utilization, CO_2 production (not shown) and ATP export.

Thus, mitochondrial metabolism possesses intrinsic satiety features that allow fuel utilization to be increased up to a point. Beyond that point, carbon inputs are converted to citrate and malate, which are exported to the cytosol for production of fat, sugar and other molecules.

Not all fuels are the same

Because the PDH complex requires both NAD^+ and FAD in oxidized forms, it is difficult for pyruvate, the carbohydrate-derived mitochondrial fuel, to saturate mitochondrial oxidation capacity on its own. For example, if NADH and $FADH_2$ produced by PDH and by oxidation of PDH-derived Ac-coA were at such great concentrations that they could not be reoxidized by the ETC, then PDH would be retarded by the tendency of NADH to bind to the NAD^+ site and the consequent persistent occupancy of $FADH_2$ at the FAD site. This would slow formation of Ac-coA, such that the ETC would be able to reoxidize NADH to NAD^+, thus restoring the oxidizing environment essential for further pyruvate utilization.

In a similar manner, there are systems that limit the ability of dietary fatty acids to saturate mitochondrial oxidative capacity. Dietary fatty acids are long chain fatty acids (LCFA), which require the carnitine shuttle system for mitochondrial import. When lipogenesis is occurring, the cytosol contains malonyl-coA, which inhibits carnitine palmitoyltransferase 1 and LCFA import (McGarry *et al.*, 1978). Thus, mitochondria that have already achieved energy satiety, that is, which are exporting citrate for production of cytosolic Ac-coA and malonyl-coA, do not import LCFA.

Short and medium chain fatty acids (SCFA and MCFA) and amino acids are not subject to these systems. Such molecules cross the mitochondrial inner membrane and gain access to mitochondrial enzymes, thereby producing Ac-coA and acetoacetyl-coA at the expense of free coA. Thus, SCFA, which are produced by the intestinal microbiome, particularly in diet-induced obesity (Turnbaugh *et al.*, 2006), and protein-rich meals may have the tendency to saturate the mitochondrial coA pool. The combination of high carbohydrate, high protein and SCFA would tend to convert free mitochondrial coA to Ac-coA very effectively, thereby

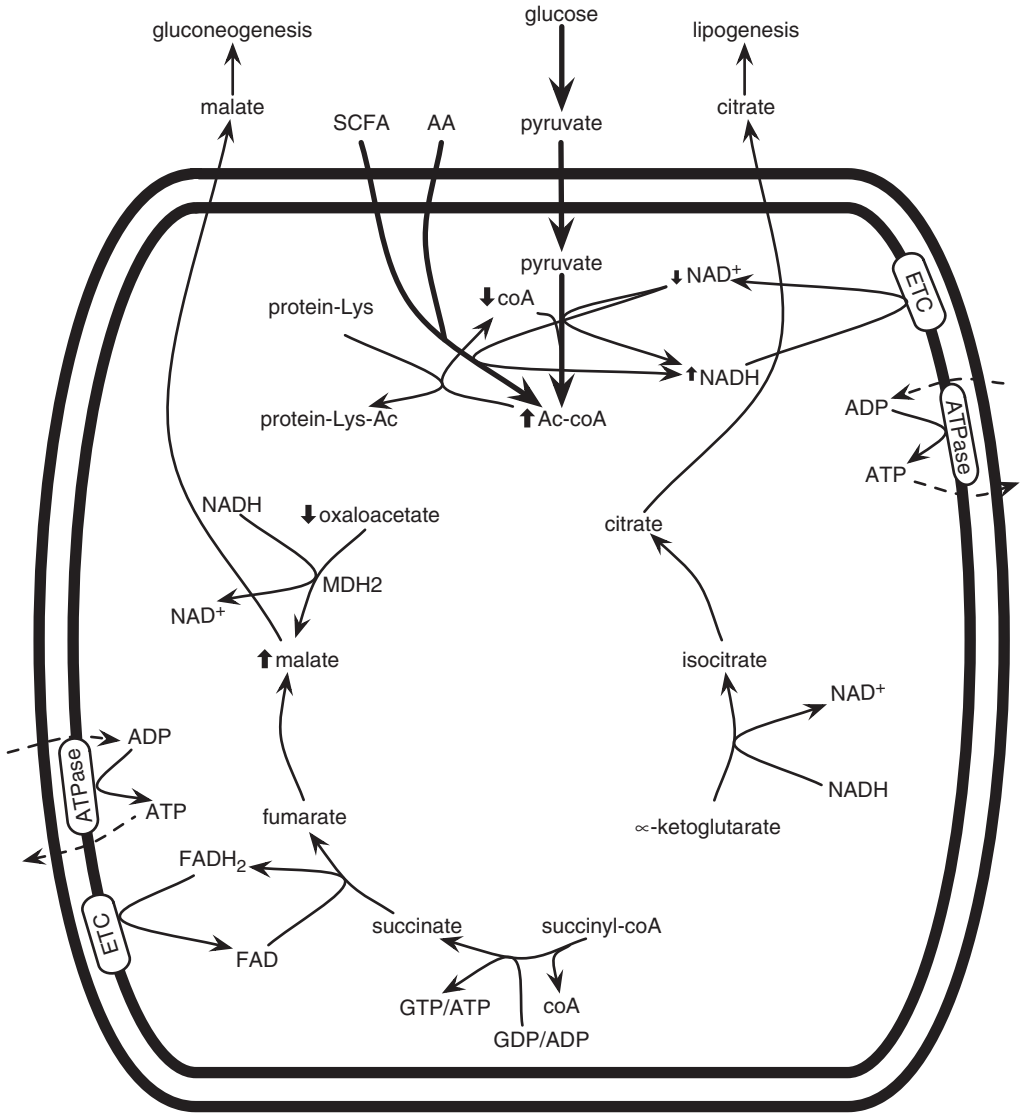

Figure 2. Mitochondrial function during macronutrient overnutrition. Here, mitochondria are importing pyruvate, short chain fatty acids (SCFA) and amino acids (AA) at rates that exceed the capacity of the ETC to reoxidize NADH. Under these conditions, mitochondrial coA accumulates in the Ac-coA form, in part, due to the inability of MDH2 to form oxaloacetate in the presence of high NADH. Without free coA for α-ketogluarate dehydrogenase (α-KGDH) to run, metabolites early in the citric acid cycle leave mitochondria as citrate, precursor for cytoplasmic Ac-coA and lipogenesis. Metabolites late in the citric acid cycle leave as malate, precursor for cytoplasmic oxaloacetate and gluconeogenesis. At high Ac-coA, protein acetylation occurs.

inhibiting citric acid cycle function at the α-KGDH complex (Figure 2).

Whether the fats are SCFA or LCFA, it is a ''red letter day'' when an animal encounters supersaturating amounts of carbohydrate, protein and fat. We term this state ''macronutrient overnutrition''. Carbon inputs to mitochondria initially cause fuel to be oxidized. At respiratory capacity, Ac-coA and NADH are expected to rise, thereby inhibiting mitochondrial fuel oxidation, and leading to export of malate and citrate. LCFA then tend to be deflected directly into lipogenesis, while SCFA, pyruvate and amino acids would tend to continue to enter mitochondria, producing a ''mitochondria are full'' metabolic signature of high Ac-coA, high NADH, high ATP and potentially high matrix pH. This metabolic signature does not require any hormonal system to produce – it is predicted to occur simply because of the finite capacity of respiratory metabolism. When mitochondria are full, citrate flows out for production of cytosolic Ac-coA and subsequent lipogenesis. The red letter day is marked by production of valuable fat stores that protect against famine.

Consequences of high mitochondrial Ac-coA include protein acetylation

Thus far, textbooks depict two fates for mitochondrial Ac-coA: oxidation by the citric acid cycle and ketone body production. Oxidation requires NAD^+, free coA, nucleoside diphosphates and oxaloacetate. Ketone body production in liver mitochondria occurs during carbohydrate shortages, largely because oxidation of fats causes a buildup of Ac-coA and a shortage of oxaloacetate, with malate being exported to the cytosol for gluconeogenesis (McGarry & Foster, 1980). Fuel oxidation and ketogenesis both relieve high Ac-coA and release free coA. However, whereas citric acid cycle oxidation occurs when there is NAD^+, free coA and oxaloacetate, ketogenesis can occur without free coA or NAD^+ in the

oxidized form. Is there another fate for mitochondrial Ac-coA, which would occur specifically under the conditions in which mitochondria are overloaded with fuel?

In vitro (Zhao *et al*., 2010) and *in vivo* (Hirschey *et al*., 2011) experiments have recently established that when fuel is increased, mitochondrial protein lysine acetylation is increased. Because the source of protein acetylation is Ac-coA (Bondy *et al*., 1970), mitochondrial Ac-coA must be considered to have a third major fate. As fuel is increased and mitochondrial Ac-coA increases, more protein Lys acetylation occurs. Such protein modifications would tend to relieve mitochondrial Ac-coA and could have important regulatory roles.

One of the first mitochondrial acetylomic analyses (Zhao *et al*., 2010) showed that metabolic enzymes are highly modified by acetylation and that acetylation is increased when fuel is increased. However, it was claimed that the hyperacetylated form of MDH2 promotes improved fuel oxidation. What the study actually shows is that MDH2 becomes acetylated at Lys185, Lys301, Lys307 and Lys314 and that enzyme activity is increased for hyperacetylated MDH2 but not for MDH2 in which the four Lys residues were replaced with Arg (Zhao *et al*., 2010). However, the fact that hyperacetylated MDH2 is more active does not support the idea that fuel oxidation is improved during such conditions. Because of the thermodynamic favorability of oxaloacetate reduction, MDH2 is assayed in the direction contrary to fuel oxidation, that is, $NADH + oxaloacetate \rightarrow NAD^+ + malate$. During conditions of high fuel (high NADH) this is not only the convenient manner to assay MDH2, it is the biologically relevant direction. This means that hyperacetylated MDH2 in the presence of high NADH would more rapidly reduce oxaloacetate to malate (and keep malate from forming net oxaloacetate), thereby opposing fuel oxidation and promoting malate export. In fact, this biochemical analysis would be consistent with a diabetogenic effect of increased fuel. Increased fuel would not promote improved fuel oxidation because of MDH2 protein acetylation: increased fuel and consequent hyperacetylated MDH2 would promote malate export and potentially an increase in gluconeogenesis and hyperglycemia.

Mitochondrial acetylation is almost always inhibitory

Although the notion that mitochondrial protein acetylation increases fuel oxidation does not have much currency, the observation that mitochondrial protein acetylation is regulatory has been proven again and again. MDH2 is an unusual enzyme that is activated by hyperacetylation. Among the mitochondrial enzymes that are inactivated by Lys acetylation are Ac-coA synthetase 2 (Hallows *et al*., 2006; Schwer *et al*., 2006), glutamate dehydrogenase (Schlicker *et al*., 2008), complex I of the ETC (Ahn *et al*., 2008) IDH2 (Schlicker *et al*., 2008; Yu *et al*., 2012), long chain acyl-coA dehydrogenase (Hirschey *et al*., 2010), 3-hydroxy-3-methylglutaryl coA synthetase 2 (Shimazu *et al*., 2010), superoxide dismutase 2 (Qiu *et al*., 2010; Tao *et al*., 2010) and ornithine transcarbamylase (Hallows *et al*., 2011). This represents a wide range of key mitochondrial functions including fuel oxidation, energy generation, waste disposal and detoxification of reactive oxygen species.

Evidence that these enzymes are inactivated by Lys acetylation is derived by knocking out Sirtuin 3 (Sirt3), a nuclear-encoded enzyme, which accounts for mitochondrial protein Lys deacetylase activity (Lombard *et al*., 2007). Multiple studies have established that mitochondrial proteins accumulate in acetylated or hyperacetylated forms in *sirt3* −/− animals (Ahn *et al*., 2008; Hallows *et al*., 2011; Hebert *et al*., 2013; Hirschey *et al*., 2010; Lombard *et al*., 2007; Qiu *et al*., 2010; Shimazu *et al*., 2010; Tao *et al*., 2010; Yu *et al*., 2012). With the exception of MDH2, which is apparently activated by hyperacetylation (Zhao *et al*., 2010), all of the characterized mitochondrial acetylation targets have lower activity in the acetyl-modified forms. Indeed, it has recently been contended that deacetylation by Sirt3 is always activating (Chhoy *et al*., 2013).

The literature tells us that Sirt3, expression of which is increased during calorie restriction (Hirschey *et al*., 2010; Palacios *et al*., 2009; Shi *et al*., 2005), activates the functions of a wide swath of mitochondrial enzymes. This is particularly interesting because Sirt3, like all sirtuins, is an NAD^+-dependent protein lysine deacetylase (Belenky *et al*., 2007a; Feldman *et al*., 2012; Sauve *et al*., 2006). However, we would suggest that because Sirt3 targets are synthesized and are presumably imported into mitochondria without Lys modifications, they are really *inactivated* by acetylation and have their activities restored by NAD^+-dependent protein lysine deacetylation.

Protein acetylation as a memory of a metabolic signature

Metabolites themselves, in particular the ratios of key metabolites, such as NAD^+ to NADH and ATP to ADP, can direct metabolic flux. However, metabolite-driven changes in metabolic flux are so instantly homeostatic that they are often reinforced by covalent modifications that create a memory of metabolic conditions. Consider a cell that is experiencing a state of anoxia, which drives down ATP formation and can be considered a state of cellular hunger for energy inputs. The initial response to severe declines in the ATP:ADP ratio is mediated by formation of AMP. The glycolytic increase, producing new ATP, that results from appearance of AMP is so instantaneous that it erases the AMP signal. Were it not for AMP-activated, kinase-mediated protein phosphorylation, there would be no lasting memory of a spike in AMP. We will draw an analogy between AMP signaling and Ac-coA signaling.

When cellular ATP is low, ADP is high. Under these conditions, adenylate kinase converts two ADP to ATP plus AMP. AMP functions as an activator of catabolism by displacing an inhibitory ATP bound to an allosteric site on phosphofructokinase 1 (PFK1) (Wegener & Krause, 2002). So long as there is AMP bound to PFK1, the enzyme is activated and flux is increased through glycolysis. However, because PFK1 activation leads to rapid conversion of glucose to pyruvate with production of net ATP, AMP formation is rapidly followed by ATP production. With rising ATP, adenylate kinase reverses its net direction by using ATP to

phosphorylate AMP, thereby erasing the initiating signal of low energy.

The instantly homeostatic nature of AMP as an allosteric regulator (i.e. AMP leads to production of ATP, which erases AMP) leaves a need for a covalent modification system that can sustain an increase in glycolysis and other responses to cellular hunger. Because AMP-activated protein kinase phosphorylates GLUT4 and phosphofructokinase 2 (PFK2), anoxic cells can ramp up glucose transport and glycolysis and sustain this flux using the fructose-2,6-bisphosphate activator of PFK1 beyond the time at which AMP is converted to ADP (Hardie, 2011). Indeed, because AMP-activated protein kinase creates covalent marks on multiple targets, a subsequent decline in glycolytic flux must await protein dephosphorylation or protein turnover. This example of the AMP metabolite directing metabolic flux through an instantly homeostatic mechanism linked to a longer lasting covalent modification system has parallels in mitochondrial Ac-coA metabolic signaling.

If AMP represents a cellular state of hunger, the metabolic signature of high Ac-coA, high NADH and high ATP represent mitochondrial overnutrition. These conditions inhibit fuel oxidation because the activities of pyruvate dehydrogenase and other NAD^+-dependent oxidation reactions are disfavored at high NADH. Citrate synthetase slows down because of the lack of oxaloacetate, and IDH runs in reverse (Des Rosiers *et al*., 1994), which leads to citrate export and lipogenesis. Under these conditions, pyruvate carboxylase is allosterically activated by Ac-coA and generates oxaloacetate from pyruvate and ATP (Jitrapakdee *et al*., 2008). However, at high NADH, the oxaloacetate is reduced to malate by MDH2, such that pyruvate carboxylase does not provide the limiting substrate for citrate synthetase. At high Ac-coA, high NADH and high ATP in the overfed liver, pyruvate carboxylase initiates gluconeogenesis.

Just as glycolysis is activated by low ATP and the presence of AMP, mitochondrial metabolism is initially self-correcting. When IDH and MDH2 run in reverse, NADH is reoxidized to NAD^+ and, as pyruvate carboxylase continues to run, high ATP can be worked off. Suddenly, pyruvate can be used by pyruvate carboxylase and PDH to make both citrate synthetase substrates and the citric acid cycle can be restarted. The ability of systems such as these to work off the ''mitochondria are full'' metabolic signature makes mitochondria resilient to transient overnutrition and is routine in the post-prandial state. However, while restoring the ability to oxidize fuel sounds appealing, it is not necessarily of the greatest evolutionary benefit to an organism.

If the ''mitochondria are full'' metabolic signature could be imprinted on mitochondrial proteins in a manner that were to retard oxidative functions more than transiently, then an organism that encounters conditions of macronutrient surplus might more effectively retard its metabolism and be able to store valuable fat. Specifically, if rising Ac-coA is converted to covalent modification of mitochondrial proteins, then protein acetylation could be an imprint of nutritional conditions that lasts longer than the metabolic signature itself.

For the vast majority of metabolic enzymes, the functional consequences of whose acetylation have been determined, acetylation is inhibitory (Ahn *et al*., 2008; Hallows *et al*., 2006, 2011; Hebert *et al*., 2013; Hirschey *et al*., 2010; Schlicker *et al*., 2008; Schwer *et al*., 2006; Shimazu *et al*., 2010; Qiu *et al*., 2010; Tao *et al*., 2010; Yu *et al*., 2012). Indeed, if one looks at the citric acid cycle, the perfect storm to retard fuel oxidation by regulatory acetylation would be to retard nearly all enzymes and accelerate MDH2 at high NADH (Zhao *et al*., 2010). This would tend to prevent oxaloacetate conversion to citrate, despite high Ac-coA, such that malate will accumulate and be transported into the cytosol. Because α-KGDH requires free coA, citric acid cycle intermediates will flow backwards from α-ketoglutarate such that citrate can leave mitochondria for lipogenesis (Figure 2).

Thus, it is reasonable to suggest that mitochondrial protein acetylation evolved as a system to imprint a memory of macronutrient overnutrition on mitochondrial enzymes – so long as the acetyl marks remain on these enzymes, fuel oxidation would be impaired and fat storage would be improved. This is the phenotype of *sirt3*−/− mice: they are hypersensitive to weight gain, insulin resistance, hyperlipidemia and fatty liver compared to wild-type animals on chronic high fat diet (Hirschey *et al*., 2011).

And the mitochondrial protein acetyltransferase is?

Mitochondrial enzymes have been found to be highly acetylated as far back as the yeast *Saccharomyces cerevisiae* (Henriksen *et al*., 2012). In a dataset of acetylated proteins from the fruitfly *Drosophila melanogaster*, Lys residues that are acetylated were found to be highly conserved between flies, worms, zebrafish and humans, and mitochondrial proteins were found to be highly enriched in acetylation (Weinert *et al*., 2011). These data suggest an ancient co-evolved system reminiscent of a protein kinase and protein kinase substrates.

Despite significant efforts to identify a sequence encoding a mitochondrial protein lysine acetyltransferase in invertebrates, none has been found. At least nine families of protein lysine acetyltransferases have been identified: HAT1, Gcn5/PCAF, MYST, p300/CBP, Rtt109, ACTR/AIB1, TAF250, ATF-2 and CLOCK (Yuan & Marmorstein, 2013). The diversity of protein lysine acetyltransferase sequences makes sequence identification of a mitochondrial protein lysine acetyltransferase challenging.

What are the possibilities to explain the origin and conservation of mitochondrial protein lysine acetylation?

(1) Mitochondrial proteins are acetylated in the cytoplasm by nucleocytoplasmic acetyltransferases and by nucleocytoplasmic Ac-coA.
(2) Mitochondrial proteins are acetylated by a known protein lysine acetyltransferase, a fraction of which is imported into mitochondria.
(3) Mitochondrial proteins are acetylated by a novel mitochondrial enzyme, not recognizable as a protein lysine acetyltransferase.
(4) Mitochondrial proteins are chemically acetylated.

The first possibility would fail to explain how mitochondrial proteins are dynamically acetylated (Zhao *et al*., 2010). However, the second and third possibilities cannot currently be excluded. In support of the second possibility, there is

recent evidence for mitochondrial forms of calmodulin-dependent kinase II (Joiner *et al*., 2012) and protein kinase A (Sastri *et al*., 2013), which were long considered to be cytosolic or nucleocytosolic enzymes, are not in Mitocarta (Pagliarini *et al*., 2008), and do not contain mitochondrial targeting sequences. The major problem with the fourth possibility, which has recently been suggested (Newman *et al*., 2012), is how to explain the remarkable propensity of mitochondrial acetylation to hit active site residues and otherwise regulate enzyme function. If mitochondrial protein acetylation is driven by simple chemistry, how can the resulting protein modification be so site-specific?

Role of Lys pK_a in nonenzymatic acetylation

Reactions that are driven by ''simple'' chemistry are controlled by concentration of reactions and also by regiospecific reactivity – the critical statistic is the concentration of the reactive species. No matter which enzyme catalyzes acetyl group transfer reaction to a Lys side chain, the ε amino group must be deprotonated to be attacked by the partially positive carbonyl carbon of Ac-coA. Without an enzyme to recognize the polypeptide and present a base to abstract a proton from the substrate Lys, most Lys ε amino groups are poorly reactive. This is because the pK_a of a nonperturbed Lys amino group is 10.4. Accordingly, near neutral pH, a typical, surface-exposed Lys spends almost 100% of the time in the protonated state and would be quite resistant to acetylation at any concentration of Ac-coA.

However, Lys residues in special locations can have pK_a values depressed into the neutral range or lower. Indeed, Lys residues within enzyme active sites and other unique locations exhibit depressed pK_a values by virtue of nearby positive charges or by desolvation. In both cases, the local environment destabilizes the protonated NH_3^+ group with respect to the neutral, lone electron pair-bearing NH_2 group.

This model has the potential to explain the site-specificity of mitochondrial protein acetylation. Although not all Lys residues at enzyme active sites have depressed pK_a values, the requirement of a depressed pK_a value for nonenzymatic acetylation would tend to enrich for active site residues. Moreover, this system would have been the subject of 500 million years of target site evolution. If increasing Ac-coA tends to lead to mitochondrial protein modification, which inhibits mitochondrial function and promotes lipogenesis during times of energy excess, then organisms with susceptible Lys residues in key mitochondrial enzymes would tend to store more fat and have improved survival and fecundity, particularly during eras of uncertain food.

Other mitochondrial Lys modifications

Although Sirt3 is responsible for mitochondrial protein deacetylation (Lombard *et al*., 2007), the mitochondrial Sirt5 isozyme reverses succinyl and malonyl modifications of Lys (Du *et al*., 2011; Peng *et al*., 2011) and the mitochondrial Sirt4 isozyme may have an enzymatic activity on a distinct modification. Although it will be hard to eliminate the possibility that specific succinyl-coA and malonyl-coA transferases exist, we suggest that mitochondrial sirtuin substrates are formed by chemical means, that is, as a function of the concentrations of the corresponding coA thioesters and a function of the concentration and reactivity of substrate Lys residues. This would also explain why multiple modifications tend to be found on the same substrate Lys residues.

Evolutionary mutability of Lys pK_a and the role of elevated matrix pH

We propose that Lys acetylation in mitochondria arose simply as a function of elevated Ac-coA and the susceptibility of Lys residues in specific locations. If a particular Lys residue on an enzyme that promotes fuel utilization had a depressed pK_a value, say to 8.4, it would tend to become acetylated during conditions of rising Ac-coA, creating a memory or imprint of high Ac-coA and retarding fuel oxidation, thereby promoting carbon export from mitochondria and the production of fat. If this were to create an evolutionary advantage, one would expect that additional enzymes would be selected to possess similar properties and that the local environment of target Lys residues could be driven to still lower pK_a values, either by nearby appearance of Lys and Arg residues or by partial desolvation. In contrast, if an enzyme essential for survival were to be susceptible to inactivation by virtue of acetylation, then evolutionary forces would tend to remove positive charges nearby, thereby elevating Lys pK_a and allowing resistance to rising Ac-coA (Figure 3).

The metabolic signature of fuel-saturated mitochondria is predicted to consist not only of high Ac-coA, high NADH and high ATP, but also elevated matrix pH. This would be the case because, at respiratory capacity, protons have been maximally pumped into the intermembrane space, thereby alkalinizing the matrix (Santo-Domingo & Demaurex, 2012). As the mitochondrial matrix pH rises, protons are abstracted from more and more Lys residues, which would tend to drive more productive reactions of Ac-coA with protein Lys residues. Thus, with persistent overnutrition, the ''mitochondria are full'' metabolic signature would be increasingly converted to high occupancy acetylation of Lys residues with the lowest pK_a values and imprinting of additional low pK_a Lys residues on abundant proteins.

Whereas protein acetylation will tend to relieve high Ac-coA and thereby allow coA-dependent enzymes such as PDH and α-KGDH to run, if acetylated proteins are largely inhibited, then a switch from carbon export/lipogenesis back to fuel oxidation has to await reversal of these modifications by the NAD^+-dependent deacetylase, Sirt3. In this manner, the high Ac-coA metabolic signature is converted to a longer lasting, covalent metabolic regulatory mechanism.

The relationship between macronutrients and micronutrients

When an animal encounters food in the wild, the energy inputs are typically living or formerly living biomass. Such ''whole foods'' can be expected to contain micronutrients in proportion to macronutrients. Because all cellular biomasses contain coA metabolites, pantothenate equivalents can be salvaged along with carbohydrate, protein and fat. Dietary pantothenate is used to generate coA, which is reportedly transported into the mitochondrial matrix by the Graves'

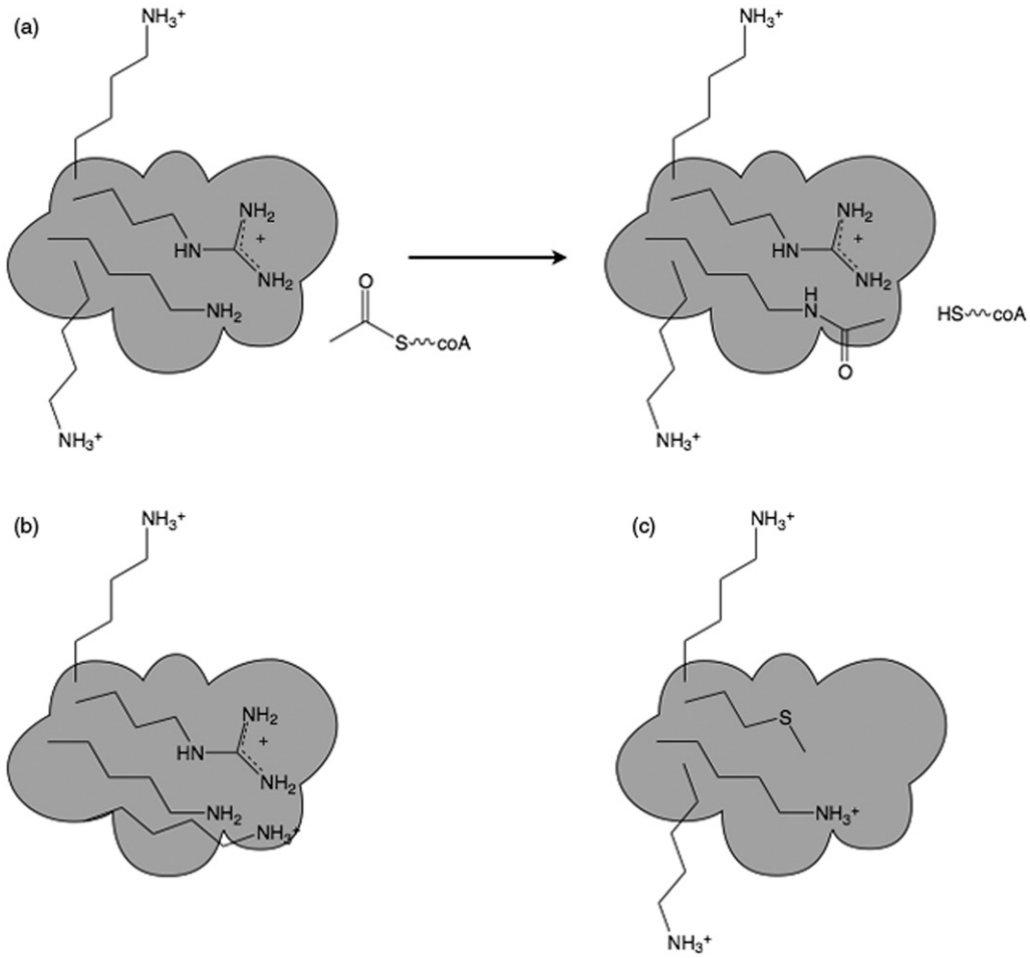

Figure 3. Chemistry and mutability of acetylation. (a) Near neutral pH, Lys residues with nonperturbed pK_a values are resistant to acetylation. One Lys near an Arg has a depressed pK_a value and is modified by Ac-coA. (b) A susceptible Lys residue has its pK_a value further depressed by virtue of accumulation of an additional nearby positive charge. This is the postulated mechanism by which regulatory Lys residues frequently acquired susceptibility to chemical acetylation. Largely, mitochondrial enzyme inactivation is proposed to be under positive selective pressure by favoring lipogenesis. (c) Here, a formerly accessible Lys residue acquires greater resistance to acetylation by virtue of loss of a nearby positive charge. This adaptive mechanism would protect against loss of functions that are essential, particularly during times of nutritional oversufficiency or undersufficiency.

disease protein (Prohl *et al*., 2001). Pantothenate deficiency could make saturation of mitochondrial coA occur at a lower level of fuel. How mitochondria regulate their total levels of coA synthesis as a function of dietary pantothenate is not known (Leonardi *et al*., 2005).

The most abundant NAD^+ metabolite is NAD^+ itself (Evans *et al*., 2010; Trammell & Brenner, 2013). During digestion, NAD^+ metabolites are broken down to the salvageable precursor vitamins, nicotinate, nicotinamide and nicotinamide riboside (NR) (Belenky *et al*., 2007b; Bieganowski & Brenner, 2004; Bogan & Brenner, 2008). Trp is also used to produce NAD^+ *via* the *de novo* pathway in the tissues in which such enzymes are expressed. Whereas any of the four NAD^+ precursors can produce nucleocytoplasmic NAD^+, Trp and nicotinic acid produce NAD^+ through the nicotinate mononucleotide (NaMN) intermediate, whereas nicotinamide and NR produce NAD^+ through the nicotinamide mononucleotide (NMN) intermediate (Trammell & Brenner, 2013). Because mitochondrial NAD^+ depends on import of NMN (Nikiforov *et al*., 2011), nicotinamide and NR are predicted to be superior to nicotinate and Trp as mitochondrial NAD^+ precursors (Trammell & Brenner, 2013). However, because nicotinamide inhibits sirtuins at high dose (Anderson *et al*., 2003), NR may be the most effective NAD^+ precursor to elevate mitochondrial sirtuin activities (Trammell & Brenner, 2013).

We have argued that mitochondrial acetylation forms a covalent memory of elevated mitochondrial Ac-coA and that such modifications are largely inhibitory for mitochondrial function. Because mitochondrial acetylation is reversed by Sirt3 in a manner that requires consumption of one equivalent of NAD^+, and NADH is not a sirtuin substrate, reoxidation of NADH and/or continual resynthesis of mitochondrial NAD^+ is required for Sirt3 activity. Thus, while mitochondrial protein acetylation is an erasable memory of overnutrition, as long as the mitochondrial NAD^+ pool is largely in the NADH form, these modifications would be difficult to erase. In addition, because Sirt3 activity cleaves NAD^+ into a nicotinamide moiety and an Ac-ADPribosyl product (Belenky *et al*., 2007a; Feldman *et al*., 2012; Sauve *et al*., 2006), Sirt3 activity without mitochondrial NAD^+ resynthesis may not effectively restore the ability to oxidize fuel. Mitochondrial NAD^+ resynthesis requires cytosolic nicotinamide phosporibosyltransferase (Revollo *et al*., 2004) and/or NR kinase (Bieganowski &

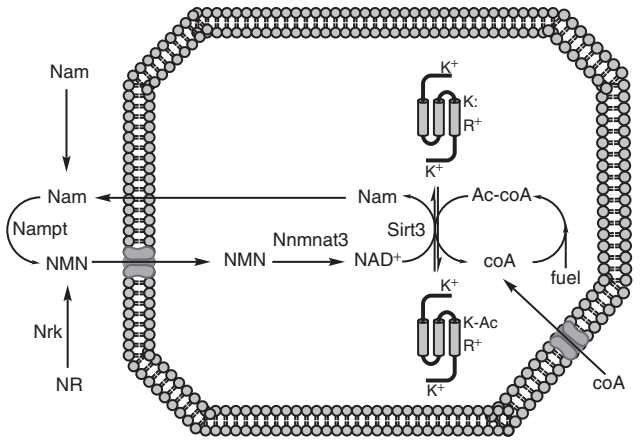

Figure 4. Relationship between co-enzymes and reversible acetylation. coA, which is synthesized from pantothenate in cytoplasm and mitochondria (not shown) is ultimately imported into mitochondria, where it becomes acetylated by pyruvate and fatty acid oxidation and amino acid catabolism. Protein acetylation releases free coA. Mitochondrial protein deacetylation depends on NAD^+ and Sirt3, producing nicotinamide (Nam) and Ac-ADP ribose (not shown) in addition to nonmodified protein. Salvage to nicotinamide mononucleotide (NMN) occurs in the cytoplasm as a function of Nam and Nam phosphoribosyltransferase (Nampt) or nicotinamide riboside (NR) and nicotinamide riboside kinase (Nrk). NMN is imported to mitochondria, where it is a substrate of NMN adenylytransferase 3 (Nmnat3).

Brenner, 2004), the corresponding NAD^+ precursor and the mitochondrial NMN adenylyltransferase, Nmnat3 (Nikiforov *et al*., 2011) (Figure 4).

Sirt3 protein expression is under the control of the nutritional status of an organism. In mice, high fat diet leads to a decline in Sirt3 protein accumulation (Hirschey *et al*., 2011; Palacios *et al*., 2009), whereas calorie restriction leads to an increase in Sirt3 expression (Hirschey *et al*., 2010; Palacios *et al*., 2009; Shi *et al*., 2005). Thus, chronic overnutrition is expected to lead to an increased rate and persistence of mitochondrial protein acetylation. Declining Sirt3 would tend to fix these inhibitory marks and lead to progressive mitochondrial dysfunction, which is frequently seen in obesity and its complications (Mantena *et al*., 2008). While the intrinsic programming that leads to reduced Sirt3 expression in overfed animals sounds like a bad thing, this is precisely the programming that would preserve fat stores and be selected by evolution, so long as it does not interfere with survival, mating or child-rearing.

The epidemic of human obesity is not associated with hyperphagy of minimally processed foods. Although our energy intake exceeds our energy expenditure, the foods we increasingly eat are those, which our food industry aims to sell (Chandon & Wansink, 2012). These are typically refined and concentrated in macronutrients. Ironically, the epidemic of pellagra of 100 years ago was traced to the diet of poor people in the American South, who ate corn rations and lard. The American snack food industry is built around similar ingredients. Although pellagra was cured with fresh foods and has been largely prevented with nicotinate and/or nicotinamide enrichment of cereals, the ~15 mg per day recommended daily allowance of this vitamin might be suboptimum today. This could be because there are lower niacin equivalents in current processed food diets; because current food consumption is greater and produces a greater protein acetylation phenotype, necessitating more NAD^+ salvage; or because people today are bigger and have a different body composition. Indeed, mice on high fat diet that were supplemented with NR were able to resist some increase in adiposity (Canto *et al*., 2012). Although many other mechanisms may be at work, this may be evidence that increased mitochondrial NAD^+ biosynthesis would tend to reduce mitochondrial protein acetylation, improve fuel utilization and resist lipogenesis. Experiments to test this hypothesis are in progress and have the potential to improve human nutrition and health in the face of macronutrient overnutrition.

According to this theory, NAD^+ precursors will only aid in deacetylating and reactivating mitochondrial function if Sirt3 is still expressed. It is therefore of interest to note that muscle Sirt3 expression has been reported to decline in older sedentary people but that Sirt3 expression remains high in physically active people independent of their age (Lanza *et al*., 2008).

Gcn5l1, a potential component of vertebrate mitochondrial protein acetylation

Gcn5 is a 439 amino acid histone acetyltransferase from *S. cerevisiae* that acetylates histone H3 on Lys14. In the context of larger enzyme complexes and nucleosomes, Gcn5 acetylates an expanded repertoire of Lys residues (Grant *et al*., 1999). Recent work identified a vertebrate-specific Gcn5-related protein, termed Gcn5l1, which consists of a 31 amino acid mitochondrial targeting sequence followed by 94 amino acids of Gcn5-like catalytic domain. Knockdown of Gcn5l1 resulted in a hypoacetylation phenotype, suggesting that it may mediate transfer of the acetyl group from Ac-coA to mitochondrial protein targets (Scott *et al*., 2012).

Because this enzyme is absent from yeast and more ancient metazoans, Gcn5l1 cannot be responsible for the ancient evolutionary history of mitochondrial protein acetylation. Moreover, the lack of protein domains for protein substrate recognition would argue against a role for Gcn5l1 in targeting specific Lys residues for modification. Rather, we suggest that vertebrates may have recruited a minimalist enzyme to catalyze acetyl group transfer and that the specificity for Lys residue modification remains a function of substrate pK_a values.

The appearance of Gcn5l1 as a candidate vertebrate mitochondrial protein acetyltransferase may constitute evidence that inhibitory hyperacetylation of mitochondrial proteins is not a flaw in vertebrate mitochondrial protein function, but rather a feature of our biology only recently exposed to an environment of persistent energy excess. Indeed, the evolutionary benefit of retarding fuel utilization and promoting fat storage when energy is in excess may have favored ancestors in which key enzyme mutations put Lys residues in harm's way by reducing pK_a and increasing susceptibility to acetylation at high Ac-coA. This process may have predated the appearance of vertebrate Gcn5l1 by hundreds of millions of years.

If mitochondrial hyperacetylation occurs during overnutrition, how can mitochondrial hyperacetylation also occur during undernutrition?

One of the paradoxes of mitochondrial protein acetylation is that not only does a high fat diet produce this phenotype

(Hirschey *et al*., 2011), but calorie restriction also induces mitochondrial protein acetylation (Hebert *et al*., 2013; Schwer *et al*., 2009). Although targets are not necessarily the same in different tissues, liver – the best studied organ for analysis of mitochondrial protein acetylation – is subject to hyperacetylation in overnutrition and in calorie restriction, which we term ''macronutrient undernutrition''. Deletion of Sirt3 and overnutrition both produce a fatty liver phenotype (Hirschey *et al*., 2011), suggesting that Sirt3 is responsible for removing acetylation marks that occur as a result of overfeeding and the ''mitochondria are full'' metabolic signature. Interestingly, the hyperacetylation signature from calorie restriction is not the same as that of Sirt3 deletion and it was confirmed that Sirt3 expression is increased upon calorie restriction (Hebert *et al*., 2013).

One way to explain this would be to invoke an overnutrition-induced mitochondrial acetyltransferase and a distinct undernutrition-induced mitochondrial acetyltransferase. This would depend on discovery of such enzymes or, potentially, different specificity factors that work with Gcn5l1. However, the chemical modification theory can also account for hyperacetylation during undernutrition and can account for the qualitative differences between accumulated acetylation marks in the two conditions.

During macronutrient undernutrition and ketogenic diets, the liver enters a ketotic state in which fatty acids and amino acids are oxidized. However, fat-derived Ac-coA cannot run a complete cycle when the malate produced is drawn off for gluconeogenesis. Under these conditions, Ac-coA from fat and muscle breakdown accumulates, but there is virtually no mitochondrial oxaloacetate. Just as carbohydrate limitation results in liver production of ketone bodies from high mitochondrial Ac-coA (McGarry & Foster, 1980), calorie restriction would elevate mitochondrial Ac-coA. Unlike the ''mitochondria are full'' metabolic signature of high Ac-coA, high NADH and high ATP, the ''mitochondria are ketotic'' signature would retain levels of NAD^+ and ADP to run the cycle but would be low in oxaloacetate (Figure 5). If mitochondrial protein acetylation is largely driven by high Ac-coA, then underfed and overfed mitochondria might

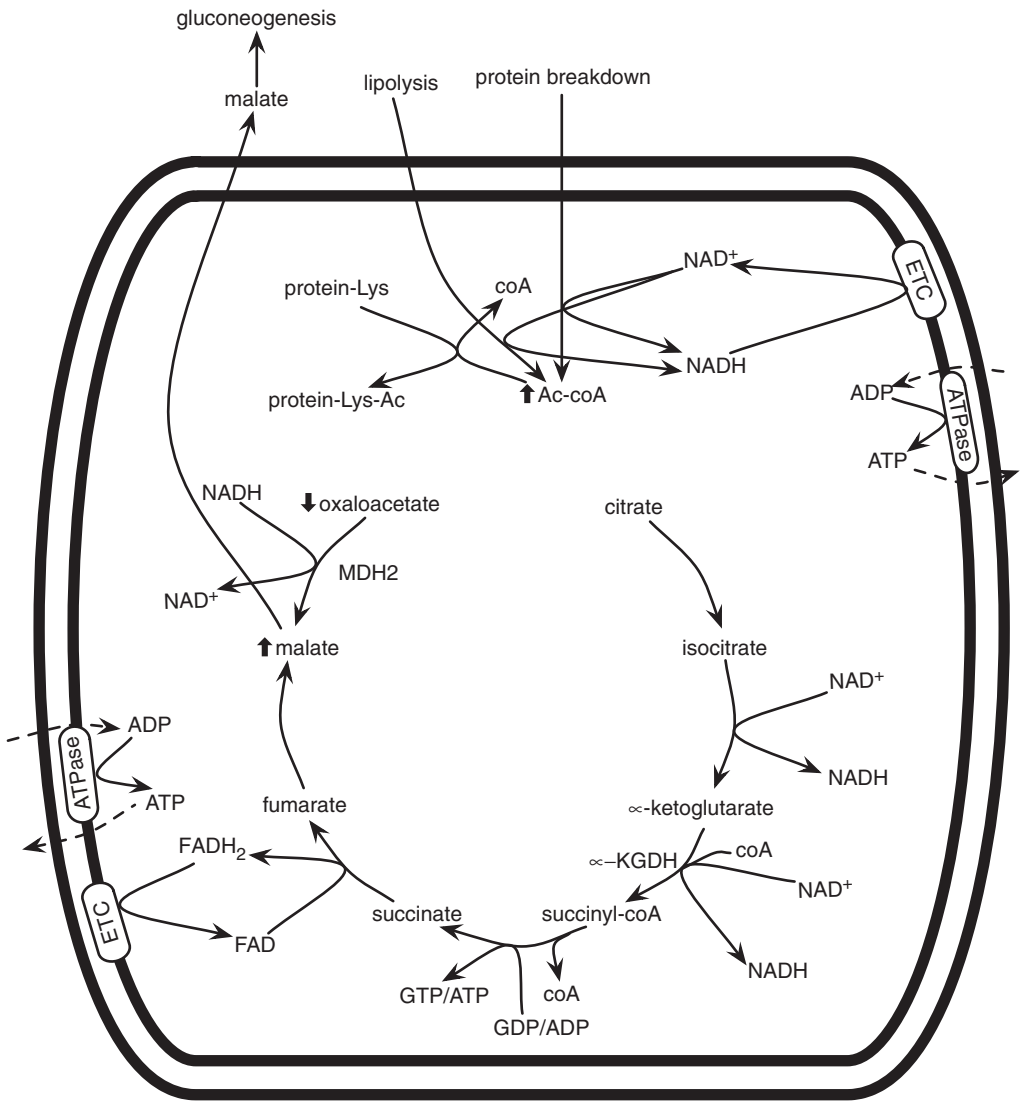

Figure 5. Mitochondrial function during macronutrient undernutrition. When carbohydrates become low, mitochondria oxidize fats and protein from bodily stores. Lipolysis and amino acid catabolism produce mitochondrial Ac-coA, which enters the citric acid cycle. However, under conditions of low blood glucose, mitochondria in gluconeogenic tissues export malate such that oxaloacetate is depleted, leading to Ac-coA accumulation. In liver, ketone bodies are produced (not shown). Elevated Ac-coA leads to protein acetylation.

exhibit similar rates of acetylation. However, because Sirt3 expression increases in response to underfeeding (Hirschey *et al*., 2010; Palacios *et al*., 2009; Shi *et al*., 2005) and decreases in response to overfeeding (Hirschey *et al*., 2011; Palacios *et al*., 2009), then acetylation during calorie restriction would be expected to be more dynamic: the net result of a high on-rate and a high off-rate. In contrast, acetylation in high fat diet-fed animals would tend not to be reversed as rapidly.

What are the specific hyperacetylation signatures of Sirt3 deletion and calorie restriction?

A recent study used quantitative acetylomic methods to characterize the liver mitochondrial proteome as a function of calorie restriction and Sirt3 deletion. Although calorie restriction increases acetylation-site occupancy of 135 acetyl sites by >2-fold when compared to the control diet, another 100 peptides exhibited decreased acetylation under calorie restriction (Hebert *et al*., 2013). This is consistent with the idea that acetylation and deacetylation are simultaneously increased in calorie restriction – the net increase in acetylation may represent the class of peptides that are relatively resistant to Sirt3 activity. The majority of sites with decreased acetylation in calorie restriction show dramatically increased acetylation (8- to 100-fold) in Sirt3 knockout animals (Hebert *et al*., 2013). These sites are apparently the ones that are readily deacetylated by Sirt3 – they may also represent the sites that would accumulate in overfed animals as the ''mitochondria are full'' signature takes hold and Sirt3 expression level declines.

It is important to remember that a protein Lys deacetylase does not control the substrates it sees. Unlike a protein kinase that is faced with various protein Ser, Thr and Tyr residues, and whose specificity is determined by its k_{cat}/K_m for each of those sites, a deacetylase encounters a protein decorated with acetylated Lys residues at particular locations. Thus, Sirt3 can erase (or not) the marks that are made but it does not have the opportunity to make any specific marks on its own. We contend that the specificity of protein acetylation – as the marks go on – is driven by chemistry and protein evolution, and that the difference in accumulated acetylation marks is a function of which sites are relatively resistant to Sirt3. Sirt3-resistant sites would accumulate during calorie restriction when Sirt3 is upregulated. Moreover, we would expect that if acetylation is driven by substrate pK_a values and nonperturbed Lys residues have pK_a values of 10.4, then undernutrition-induced acetylation sites will have evolved in the same way as overnutrition-induced sites: by providing a fitness benefit to the organism to become modified in conditions of elevated Ac-coA.

Despite the tendency of mitochondrial protein acetylation to hit active site residues and impair enzyme function, we contend that this provides a fitness benefit to overfed organisms because covalent modification of mitochondrial function would last longer than the transient increase in mitochondrial Ac-coA. In the case of overnutrition, this would tend to shunt citrate to the cytoplasm to promote lipogenesis. In the case of undernutrition, mitochondrial Ac-coA will rise due to a shortage of oxaloacetate, but fasting conditions would promote lipolysis and muscle wasting to provide malate and glucogenic amino acids for blood glucose. As soon as carbohydrates become available, one would expect that citric acid cycle intermediates would be replenished and complete Ac-coA oxidation would be restarted. However, if mitochondrial Ac-coA acetylates the mitochondrial proteome, then respiration in the tissues of a calorie restricted individual would tend to stay slow over the period of time required to remove the acetyl marks. Thus, just as AMP-dependent protein phosphorylation creates a covalent memory of cellular hunger, which lasts longer than the initiating AMP signal, mitochondrial protein acetylation is postulated to create a memory of overnutritional and undernutritional imbalances that are initially signaled by high Ac-coA and low oxaloacetate.

The evolutionary logic of retarding mitochondrial function in the overfed individual is to maintain lipogenesis while the surfeit of food winds down. The evolutionary logic of retarding mitochondrial function in the underfed individual is to slow metabolism even when food reappears. In both cases, though acetyl groups appear to be wasted by virtue of attaching them to protein side chains, the anticipated result is overall resource conservation that can maintain viability. Accordingly, this model views undernutrition-associated and overnutrition-associated acetylation sites as largely inhibitory modifications that promote survival of an organism even though mitochondrial function is attenuated.

There are two ways to depress the pK_a of a Lys. Positive charges can be located proximal to the Lys or the Lys amino group can be desolvated.

Remarkably, by quantitative analysis, Sirt3-responsive sites, which one would predict to accumulate during overnutrition, are overrepresented with Arg, Lys and Lys in positions 1, 2 and 3 amino acids carboxyl to the acetyl modification, respectively. Moreover, these sites are overrepresented on α-helices (Hebert *et al*., 2013). Placement of a positive charge one helical turn away from Lys is an excellent way to depress the Lys pK_a. Because of their depressed pK_a values, such sites would be expected to be modified at times of elevated mitochondrial Ac-coA and to further accumulate in modified forms when Sirt3 is genetically deleted or declines due to overfeeding.

Just as strikingly, the calorie restriction-induced sites were found to be located in hydrophobic sequences predicted to be inaccessible to Sirt3 (Hebert *et al*., 2013). These Lys residues potentially have their pK_a values depressed due to partial desolvation (Isom *et al*., 2011). We predict that such lone pair-bearing Lys residues are reactive with small molecules such as Ac-coA and acyl-coAs but, because of their partially buried locations, do not permit enzymes such as Sirt3 to relieve acetylation/acylation (Figure 6).

Precedents for high occupancy chemical modification

Biochemists have been trained to think that all important reactions in biology are enzyme catalyzed. However, the nonenzymatic glycosylation, termed glycation, of hemoglobin is site-specific and occurs at occupancy of 5% to 15%, depending on blood glucose concentration, in human beings. More than 20 years ago, the site specificity of hemoglobin

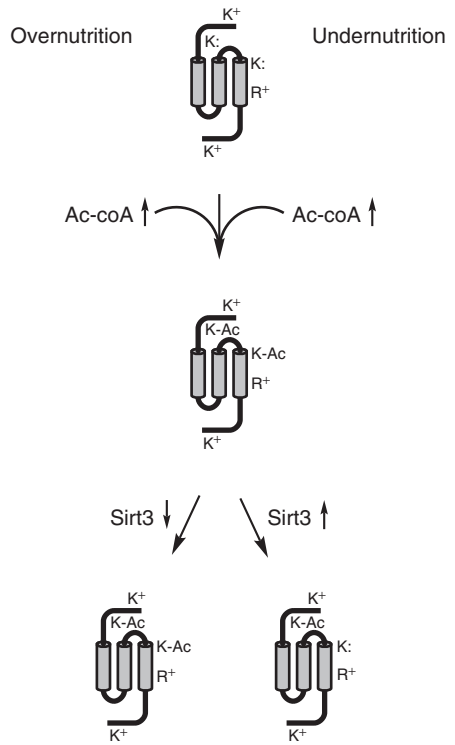

Figure 6. Net acetylation during macronutrient overnutrition and macronutrient undernutrition. As depicted in Figures 2 and 5, overnutrition and undernutrition can produce high mitochondrial Ac-coA and protein acetylation of susceptible Lys residues. In this schematic, two of four Lys residues have depressed pK_a values, depicted as K: to represent the lone electron pair of a susceptible amino group. Two Lys residues without perturbed pK_a values and an Arg are depicted as positively charged. One susceptible Lys is on an α-helix, one turn away from Arg. The other susceptible Lys is buried and partially desolvated. Under conditions of overnutrition, Sirt3 expression declines, such that both classes of acetylation are sustained. Under conditions of undernutrition, Sirt3 expression increases, leading to deacetylation of the exposed but not the buried Lys residue.

glycation was shown to vary with the pK_a of the reactive amino groups (Acharya *et al*., 1991). More recently, 1,3-bisphosphoglycerate has been shown to chemically modify lysine residues in glycolytic enzymes in a manner that depends on target site reactivity and that retards glycolytic flux (Moellering & Cravatt, 2013).

Conclusions

Macronutrient undernutrition and overnutrition can both produce mitochondrial metabolic profiles that elevate Ac-coA. Whereas Ac-coA and oxaloacetate initiate the citric acid cycle, nutritional imbalances tend to deplete oxaloacetate either because it is needed for gluconeogenesis or because the overfed mitochondria are too reducing. Because the mitochondrial coA pool is saturable, undernutrition conditions will tend to drive Ac-coA formation from fat and protein stores, whereas overnutrition conditions will tend to drive Ac-coA formation from ingested macronutrients. Though the citric acid cycle cannot run without oxaloacetate or free coA, mitochondrial metabolism would bear no memory of recent nutritional imbalances without the formation of covalent impressions of these metabolic states. Discovery of mitochondrial protein acetylation that is caused by episodes of undernutrition and overnutrition prompted us to consider how such phenomena evolved, particularly without any reported evidence of mitochondrially localized protein Lys acetyltransferases in yeast or invertebrates. Here, we have proposed that modern organisms have mitochondrial enzymes with a number of Lys residues that were subject to selective pressure to depress pK_a in order to render such residues sensitive to elevated Ac-coA. This mechanism would tend to slow the recovery of mitochondrial function to returning nutrients for organisms experiencing undernutrition and maintain a state of lipogenesis in organisms that have experienced overnutrition. In both cases, efficient mitochondrial reactivation is expected to depend on the activity of Sirt3 and mitochondrial NAD^+ synthesis from a precursor such as NR that resupplies the mitochondrial NAD^+ precursor, NMN, and which does not inhibit sirtuins.

Significantly, diets that are rich in processed foods may be relatively deficient in NAD^+ precursors. Individuals who practice a ketogenic diet and individuals whose energy intake chronically exceeds their energy expenditure might be impairing mitochondrial function if their mitochondrial proteomes are heavily acetylated and acylated by related modifications. Such people would be expected to benefit from supplementation with NAD^+ precursors to promote the deacetylation and activation of mitochondrial functions. In the case of individuals who are in chronic macronutrient overnutritional states, an increase in the mitochondrial pools of Ac-coA and NAD^+ might also contribute to increased fuel oxidative capacity independent of sirtuin functions, simply by elevating levels of coenzymes to activate fuel inputs and transfer reducing equivalents through the ETC.

This thesis – that animals have evolved mechanisms that impair mitochondrial function in order to preserve body mass – has struck some as contradictory. How can an evolved system impair something as central as mitochondrial function, including fuel oxidation and the ability to detoxify reactive oxygen species? Indeed, it is easy to cite data, which suggest that the epidemic of obesity will lead to a decline in life expectancy (Olshansky *et al*., 2005), which is clearly a measure of fitness, though not reproductive fitness. However, the thesis is based on the long evolutionary history of animal evolution, principally in environments of scarcity. The current environment of nutritional excess is not a driver of the evolution of lysine pK_a values in mitochondrial proteins. The current environment is not one to which people or our companion animals are well adapted.

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Declaration of interest

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