Deregulated Coenzyme A, Loss of Metabolic Flexibility and Diabetes

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Abstract

Coenzyme A (CoA) is an essential cofactor that is emerging as a global regulator of energy metabolism. Tissue CoA levels are tightly regulated and vary in response to different conditions including nutritional state and diabetes. Recent studies reveal the ability of this cofactor to control the output of key metabolic pathways. CoA regulation is important for the maintenance of metabolic flexibility and glucose homeostasis.

Introduction

CoA is an essential and universally distributed cofactor that plays a central role in energy metabolism. This molecule contains an active thiol moiety that forms a thioester bond with cellular organic acids and acts as the major acyl group carrier. CoA participates in both the synthesis and degradation of all major fuels in the body including lipids and carbohydrates [1]. However, control of intermediary metabolism by this cofactor goes beyond its ability to activate and deliver substrates for hundreds of reactions. Several acyl-CoAs produced as metabolic intermediates allosterically modulate the activity of key enzymes and transcription factors to control flux through specific pathways [2,3]. For example, the ability of malonyl-CoA to potently inhibit carnitine palmitoyltransferase 1 (Cpt1) and fatty acid β-oxidation during lipogenesis, avoids a futile and wasteful cycle of fatty acid synthesis and degradation [3]. Acetyl-CoA occupies a strategic position at the intersection of both anabolic and catabolic pathways within the tricarboxylic acid cycle where it gauges the energy state of the cell and directs the carbon flow [4]. At the same time, acetyl-CoA is the source of the acetyl groups that covalently modify thousands of enzymes, transcription factors and chromatin to modulate their function. Thus, regulation by acetyl-CoA-dependent acetylation coordinates these different activities with cell metabolism [5-7]. CoA levels are themselves regulated and

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yet flexible to enable adaptation to the metabolic state in response to environmental changes or stimuli. CoA levels are maintained between upper and lower threshold values and vary between these thresholds in response to hormones, diet, drugs, the nutritional state and diabetes [1]. The best characterized mechanism of modulating CoA is through regulation of the biosynthetic pathway at the pantotohenate kinase step. Pantothenate kinase (PanK) catalyzes the phosphorylation of pantothenic acid to phosphopantothenic acid, and feed-back inhibition of this reaction by free CoA and CoA thioesters controls the gross output of the entire pathway [8,9].

Metabolic flexibility is defined as the ability to produce energy and sustain cellular function by adapting the utilization of nutrients to their availability. This term was originally coined by Kelley and Mandarino [10] to describe the ability of skeletal muscle in lean healthy subjects to switch from the preferential uptake, storage and degradation of carbohydrates in the fed state to high rates of fatty acid uptake and oxidation in the fasted state, otherwise known as "fuel switching". During fasting, blood glucose levels fall, while free fatty acids are released from the adipose tissue stores and become a more available energy source for metabolically flexible organs and tissues. The preferential oxidation of fatty acids, instead of glucose, spares glucose for use by the brain. The liver plays an essential role in maintaining whole-body glucose homeostasis during a fast [11]. Liver releases glucose into the blood stream by hydrolyzing its glycogen stores during fasting, and also by synthesizing glucose de novo via gluconeogenesis. In this review we focus on the evidence showing that the ability to adjust CoA levels in the liver is important to maintain the metabolic flexibility of this organ and whole-body glucose homeostasis.

CoA, metabolic flexibility and glucose homeostasis: the link between fatty acid oxidation and glucose production

Mammals possess four PanK isoforms and two of them, PanK1α and 1β, are encoded by the *Pank1* gene. *Pank1* transcripts are highly abundant in the liver, and the hepatic CoA content increases in the fasted state [12,13]. Mice lacking *Pank1* exhibit a 40% reduction in hepatic CoA which does not respond to fasting with the characteristic increase observed in wild-type mice [14]. This impairment, in turn, reduces the capacity for β-oxidation which heavily relies on the availability of unacylated CoA (CoASH) to degrade fatty acids. Thus, PanK1 is important to support the metabolic flexibility of the liver and its adaptation to the fasted state. Reduced hepatic CoA and fatty acid oxidation in the *Pank1*$^{-/-}$ livers are additionally associated with fasting hypoglycemia. This phenotype is milder than the dramatic hypoglycemia observed in mice treated with an inhibitor of all the PanK isoforms [15], and adds to the evidence that supports the tight connection between hepatic CoA and glucose homeostasis. Indeed, further analysis revealed that decreased de novo glucose production by the *Pank1*$^{-/-}$ livers is the cause for the fasting hypoglycemia of the animals, and led to the identification of CoA as a cofactor that links fatty acid degradation to gluconeogenesis (**Fig. 1A and 1B**). β-oxidation produces acetyl-CoA and NADH which, in turn, provides reducing equivalents for the synthesis of ATP. Both NADH and ATP are directly required for gluconeogenesis, which is additionally stimulated by acetyl-CoA through the allosteric activation of pyruvate carboxylase, a mitochondrial enzyme that catalyzes the first reaction

in the pathway. Although acetyl-CoA was not directly measured in our study, the significant reduction in global mitochondrial protein acetylation observed in fasted $Pank1^{-/-}$ livers suggests that the acetyl-CoA concentration in this subcellular compartment is lower, thus preventing full activation of pyruvate carboxylase and of the gluconeogenic pathway (**Fig. 1C**). Finally, it is important to mention that no differences in the activation of the transcriptional program that accompanies the transition from the fed to the fasted state are observed in the $Pank1^{-/-}$ livers, indicating that decreased levels of a key small molecule like CoA is sufficient to modify the output of metabolic pathways.

$Pank1^{-/-}$ mice contain a global deletion of the gene, and two additional features related to fuel utilization in these animals are noteworthy. First, circulating fasting ketone bodies in the $Pank1^{-/-}$ mice are similar to those measured in wild-type controls, and actually tend to be higher, in spite of the reduction in hepatic fatty acid oxidation [14]. Ketone bodies are synthesized from acetyl-CoA in the liver, and their blood level is commonly considered an indirect measurement of hepatic acetyl-CoA and fatty acid β-oxidation during a prolonged fast. The steady state concentration of ketone bodies in the blood depends on the rates of synthesis in the liver and degradation in extrahepatic tissues. The lack of $Pank1$ in tissues other than the liver could be responsible for the decreased CoASH-dependent utilization of ketones. This is more clearly observed in double knockout mice lacking both $Pank1$ and $Pank2$ that exhibit a substantial accumulation of serum ketone bodies and a more severe hypoglycemic phenotype compared to the $Pank1^{-/-}$ mice [16]. Second, $Pank1^{-/-}$ mice exhibit a significant reduction in exercise capacity compared to controls when tested for their ability to run on a treadmill until exhaustion (R. Leonardi and S. Jackowski, unpublished data). Skeletal muscle adjusts fuel selection between carbohydrates and lipids according to the intensity and duration of the exercise it is required to perform. The muscle from $Pank1^{-/-}$ mice may be unable to switch fuels efficiently because of lower CoASH and impaired fatty acid β-oxidation capacity during exercise. More studies will be required to fully characterize this additional aspect of the $Pank1^{-/-}$ metabolic phenotype.

$PANK1$, CoA and insulin

An association between insulin levels and single nucleotide polymorphisms (SNPs) on chromosome 10 in an intronic region of the human $PANK1$ gene was recently identified in a genome-wide association study (GWAS) on specific metabolic traits in the Northern Finland Birth Cohort 1966 [17]. The enrolled participants were born in 1966 in a genetically isolated region to eliminate differences in age and provide a relatively homogeneous genetic background. This study analyzed the influence of environmental exposures over several decades and genetic variation on nine metabolic traits that represent heritable risk factors for cardiovascular disease and type 2 diabetes. The parameters were serum triglycerides, high-density lipoprotein, low-density lipoprotein, glucose, insulin, C-reactive protein, body mass index (BMI) and systolic and diastolic blood pressure. Analysis of about 4200 people revealed that fasting insulin levels were exclusively associated, after correction for the BMI, with two SNPs in $PANK1$, rs11185790 and rs1075374. All other traits were associated with SNPs in 3-7 genes. Both SNPs are located in an intronic region of $PANK1$ and the effects of these mutations on $PANK1$ expression are currently not known. However, this GWAS suggested that insulin levels would be altered in $Pank1^{-/-}$ mice and, indeed, $Pank1$

deficiency causes a 50% reduction in fasting blood insulin. The $Pank1^{-/-}$ mice become hypoglycemic during fasting and the reduction in circulating insulin is likely an adaptive response of the pancreatic β-cells to prevent a more severe drop in fasting blood glucose. The combination of hypoglycemia and hypoinsulinemia is often associated with increased insulin sensitivity and improved responses to a bolus of glucose (glucose tolerance test, GTT) or insulin (insulin tolerance test, ITT) [18-20]. Glucose levels during GTT and ITT fall faster in $Pank1^{-/-}$ mice compared to control mice, indicating improved insulin sensitivity. These data extend the association between $Pank1$ expression, and glucose metabolism to include insulin homeostasis.

Deletion of *Pank1* in diabetic mice and paradigms challenged

Type 2 diabetes is a complex, chronic, multiorgan disease characterized by abnormally high blood glucose levels. The etiology of type 2 diabetes is multifactorial with strong genetic and lifestyle components and, although the pathogenesis of type 2 diabetes is not fully understood, three key defects are responsible for the appearance of the hyperglycemia: impaired insulin action, hyperinsulinemia followed by reduced insulin secretion, and increased hepatic glucose production [21,22]. $Lep^{-/-}$ (*ob/ob*) mice are deficient in the hormone leptin and are an established model for human type 2 diabetes. These animals are hyperphagic, obese and exhibit hyperglycemia, hyperinsulinemia, insulin resistance and increased gluconeogenesis [23]. Liver and skeletal muscle from $Lep^{-/-}$ animals in the fed state contain almost twice the amount of CoA found in nonobese ($Lep^{+/+}$ or $Lep^{+/-}$) littermate controls [24]. The abnormally high liver CoA content results from higher PanK activity and reduced CoA degradation through Nudt7, a nudix hydrolase that specifically degrades CoA [25]. *Nudt7* expression is significantly reduced in fed $Lep^{-/-}$ livers. While fasting of the non-obese controls is characterized by a rise in both hepatic and muscle CoA, this flexibility is lost in the $Lep^{-/-}$ mice and in the closely related *db/db* mice [26]. The observation of higher hepatic CoA and deregulated gluconeogenesis in the $Lep^{-/-}$ mice led us to test the hypothesis that deleting $Pank1$ in the leptin-deficient background might improve the diabetic phenotype. $Pank1^{-/-} Lep^{-/-}$ (dKO) mice were derived by mating $Pank1^{-/-}$ and $Lep^{+/-}$ mice [24]. CoA levels, mitochondrial β-oxidation and gluconeogenesis are significantly decreased in the livers of the dKO mice compared to the $Pank1^{+/+} Lep^{-/-}$ controls, leading to a dramatic reduction in blood glucose both in the fed and in the fasted state (**Fig. 2A**). Additionally, correction of the fasting hyperglycemia reduced the insulin levels (**Fig. 2B)** and altered fuel utilization at the whole-body level. Serum acylcarnitine profiling and indirect calorimetry show that the dKO mice have reduced metabolism of fatty acid and amino acids, while relying more heavily on carbohydrates for energy production. This is likely a metabolic adaptation to the significant reduction in blood glucose and insulin since glycogen stores are significantly depleted in skeletal muscle from fasted dKO mice. The dramatic improvement in hyperglycemia and hyperinsulinemia resulting from $Pank1$ deletion occurs in spite of persistent obesity, fatty liver and insulin resistance in both liver and muscle.

Obesity has long been associated with insulin resistance, as accumulation of toxic lipids in insulin-sensitive organs like skeletal muscle and liver has been shown to interfere with glucose uptake and insulin signaling [27,28]. In the liver, insulin resistance is manifested as

a defect in insulin-stimulated glycogen synthesis and an abnormally high rate of gluconeogenesis which, in turn, is a major contributor to the elevated fasting blood glucose observed in diabetic patients. Although it remains unclear whether hepatic steatosis preceeds insulin resistance or whether fatty liver invariably leads to impaired insulin action [29,30], strategies that reduce hepatic lipogenesis effectively correct steatosis and insulin resistance [28]. Deletion of *Pank1* in the leptin-deficient background has no effect on obesity, hepatic steatosis or insulin resistance, supporting the conclusion that reduced hepatic CoA is sufficient to restrict de novo glucose production independent of insulin signaling. Thus, *Pank1* deletion effectively uncouples obesity and/or fatty liver-associated insulin resistance from hyperglycemia. The contribution of fatty acid β-oxidation to hepatic insulin resistance, if any, is still unclear. Stimulation of β-oxidation through genetic manipulations or the use of small molecules results in improved insulin sensitivity and reduced blood glucose in models of obesity-induced diabetes [31-34]. On the other hand, the phenotype of the dKO mice clearly shows that two important aspects of the diabetic state, hyperglycemia and hyperinsulinemia, are similarly improved by inhibition of the β-oxidation capacity of the liver.

Concluding remarks

CoA is emerging as a global regulator of energy metabolism whose levels are carefully controlled and modulated to support metabolic flexibility. Indeed, adaptation of the liver to the fasted state is dependent upon a significant increase in CoA levels to sustain the high rates of fatty acid oxidation and glucose production required to maintain whole-body glucose homeostasis. Conversely, metabolic flexibility and glucose regulation are lost in diabetes, and this state is associated with abnormally high and deregulated CoA levels in both liver and muscle. Importantly, reduction of CoA synthesis primarily in the liver is sufficient to correct the hyperglycemia and hyperinsulinemia of diabetic mice in spite of persistent insulin resistance. Thus, CoA levels can regulate the output of pathways like fatty acid β-oxidation and gluconeogenesis independent of insulin signaling, transcription factors and metabolic enzymes.

Acknowledgments

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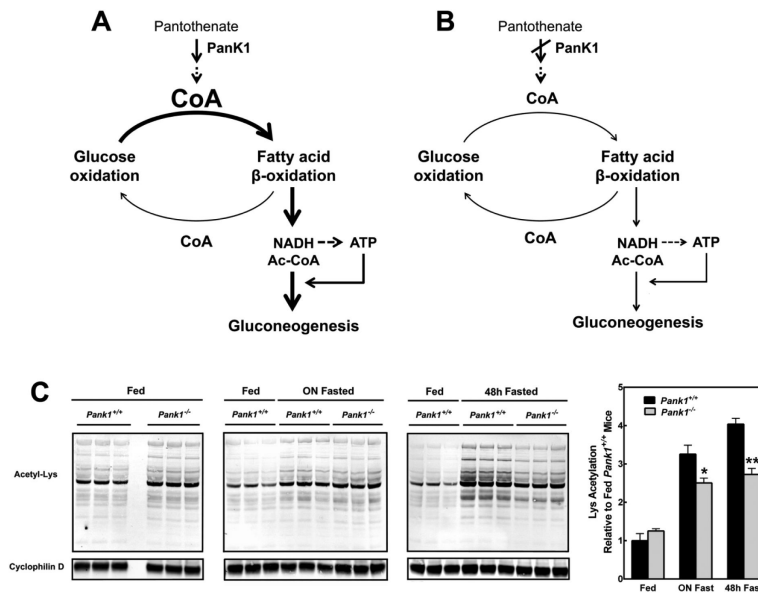
Figure 1.
CoA links between fatty acid β-oxidation to gluconeogenesis. (A) In wild-type livers, the transition from the fed to the fasted state is characterized by a significant increase in CoA levels. This increase is required to support the switch from glucose to fatty acid oxidation that occurs during a fast. Fatty acid oxidation, in turn, feeds the gluconeogenic pathway by supplying NADH, ATP and acetyl-CoA (Ac-CoA), the allosteric regulator of pyruvate carboxylase. (B) *Pank1* deficiency prevents the increase in hepatic CoA that occurs in the fasted state, thus decreasing the fatty acid β-oxidation capacity of the liver and the glucose output. (C) Global protein acetylation in liver mitochondria from fed and fasted *Pank1*^{+/+} and *Pank1*^{−/−} mice was measured by western blotting and fluorescent detection using an anti-acetyllysine antibody and cyclophilin D as a loading control. Global protein acetylation increases with fasting in *Pank1*^{+/+} but not in *Pank1*^{−/−} mice, consistent with the decrease in fatty acid β-oxidation and acetyl-CoA production caused by *Pank1* deficiency. The immunoblot fluorescence signal was quantified using a Typhoon imaging system, and normalized to the loading control. Data are reported relative to the mean normalized signal of fed *Pank1*^{+/+} mice. * $p < 0.05$, ** $p < 0.01$
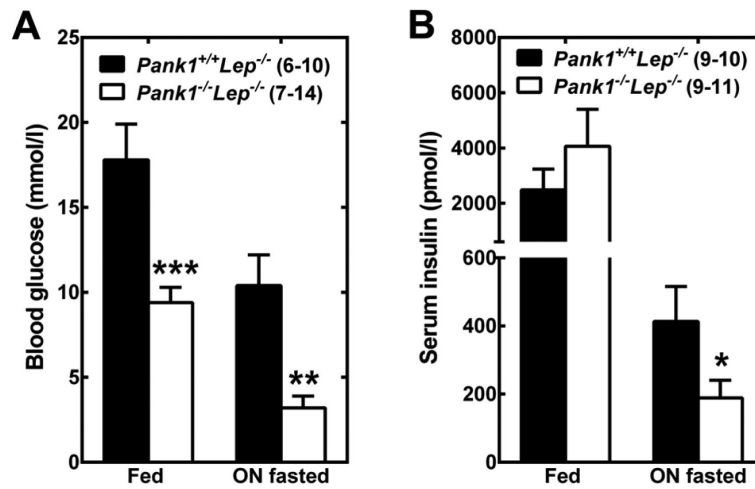
Figure 2.
Deletion of *Pank1* in the *Lep*$^{-/-}$ mice dramatically decreases hyperglycemia and hyperinsulinemia. Blood from 10-16 week-old male mice in the fed state or after an overnight (ON) fast was analyzed for glucose (A) and insulin (B) levels. The number of mice used for the measurement is indicated in brackets. * $p<0.05$, ** $p<0.01$, *** $p<0.001$. Animal handling procedures were approved under protocol 323 by the St. Jude Institutional Animal Care and Use Committee.