Minireview

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The Key Role of Anaplerosis and Cataplerosis for Citric Acid Cycle Function*

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The oxidation of acetyl-CoA to CO_2 by the TCA[1] cycle is the central process in energy metabolism. However, the TCA cycle also functions in biosynthetic pathways in which intermediates leave the cycle to be converted primarily to glucose, fatty acids, or non-essential amino acids. If TCA cycle anions are removed from the cycle they must be replaced to permit its continued function. This process is termed *anaplerosis*. Pyruvate carboxylase, which generates oxalacetate directly in the mitochondria, is the major anaplerotic enzyme. Conversely, 4- and 5-carbon intermediates enter the TCA cycle during the catabolism of amino acids. Because the TCA cycle cannot fully oxidize 4- and 5-carbon compounds, these intermediates must be removed from the cycle by a process termed *cataplerosis*. Cataplerosis may be linked to biosynthetic processes such as gluconeogenesis in the liver and kidney cortex, fatty acid synthesis in the liver, and glyceroneogenesis in adipose tissue. Cataplerotic enzymes present in many mammalian tissues include P-enolpyruvate carboxykinase (PEPCK), glutamate dehydrogenase, aspartate aminotransferase, and citrate lyase. In this review we have evaluated the roles of anaplerosis and cataplerosis in whole body metabolism.

Biochemical Role of Anaplerosis and Cataplerosis in Function of TCA

The expression *anaplerotic sequences* was a term used in biochemistry by Sir Hans Kornberg (1) to describe a series of enzymatic reactions or pathways that replenish the pools of metabolic intermediates in the TCA cycle. These intermediates are critical for the functioning of the TCA cycle, the primary role of which is the oxidation of acetyl-CoA to carbon dioxide. The pool of TCA cycle intermediates is sufficient to sustain the oxidative carbon flux over a fairly wide range, so that during high energy consumption (*e.g.* exercise) or during lower energy consumption (*e.g.* fasting), there is not a large change in the pool size of TCA intermediates (2). However, in several physiological states, there is a large influx (anaplerosis) of 4- and 5-carbon intermediates into the TCA cycle. Because the TCA cycle cannot act as a carbon sink, anaplerosis must be coupled with the exit of intermediates from the cycle via cataplerosis. The importance of anaplerotic reactions for cellular metabolism is thus apparent. However, the coupling of this process with cataplerosis and the roles that both pathways play in the regulation of amino acid, glucose, and fatty acid metabolism have not been emphasized to a sufficient extent.

The terms anaplerosis and cataplerosis describe reciprocal and correlative reactions involved in the function of the TCA cycle. The enzymatic steps in these processes have long been known, but the overall concept of a linkage between anaplerosis and cataplerosis should be underscored, because the balance between these two processes controls the entry and exit of TCA cycle anions. Anaplerotic and cataplerotic reactions are involved in the ultimate disposal of all metabolic intermediates. The metabolic role of anaplerosis and cataplerosis in amino acid metabolism varies with specific organs and is dependent on the nutritional/metabolic status of the individual. During feeding, the intestine is an important site of catabolism of enterally derived amino acids, whereas in the starved state amino acid catabolism occurs primarily in the kidney, liver, and muscle.

The catabolism of amino acids produces gluconeogenic or ketogenic precursors (Table I). The disposal of gluconeogenic anions in the TCA cycle employs anaplerotic and cataplerotic pathways for their terminal oxidation. The only known pathway for the terminal oxidation of leucine is through acetoacetate to acetyl-CoA and subsequent oxidation in the TCA cycle. However, other amino acids also have for their disposal alternate ketogenic pathways for terminal oxidation. Thus, the ketogenic amino acids from proteolysis can be terminally oxidized in muscle, whereas the gluconeogenic amino acids are dependent upon anaplerosis and cataplerosis for conversion to glucose in the liver and kidney before oxidation to CO_2 and H_2O.

Anaplerosis—The first reaction of the TCA cycle, citrate synthase, catalyzes the condensation of oxalacetate with acetyl-CoA; the oxalacetate is subsequently regenerated by the reactions of the cycle and condenses with another molecule of acetyl-CoA. However, the TCA cycle also functions in biosynthetic processes in which intermediates are removed from the cycle; this necessitates anaplerotic reactions to replenish TCA cycle intermediates to ensure its continued function. Pyruvate carboxylase, which synthesizes oxalacetate from pyruvate in the mitochondrial matrix, is the archetypical anaplerotic enzyme. The activity of this enzyme is high in many tissues (*e.g.* 10–12 units/g of liver); acetyl-CoA is a positive allosteric regulator of the enzyme. Anaplerosis is obligatory during both gluconeogenesis and lipogenesis when malate (gluconeogenesis) or citrate (lipogenesis) leaves the mitochondria and is further metabolized to form glucose or fatty acids, respectively.

Cataplerosis—If intermediates can be added to the TCA cycle, it is equally important to remove them to avoid the accumulation of anions in the mitochondrial matrix. Cataplerosis describes reactions involved in the disposal of TCA cycle intermediates. There are several cataplerotic enzymes; these include PEPCK, aspartate aminotransferase, and glutamate dehydrogenase. Each of these reactions has as substrate a TCA cycle anion that is converted to a product that effectively removes intermediates from the cycle. In the liver and kidney, the role of PEPCK in cataplerosis is of special importance

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[1] The abbreviations used are: TCA, tricarboxylic acid cycle; PEPCK, phosphoenolpyruvate carboxykinase; PEP, phosphoenolpyruvate.

TABLE I
Metabolic fates of amino acids in the TCA cycle

Pyruvate can enter the TCA cycle after being carboxylated to oxaloacetate via pyruvate carboxylase (anaplerosis). Malate synthesized from the oxaloacetate exits the TCA cycle for gluconeogenesis. Pyruvate may also be decarboxylated to acetyl-CoA by pyruvate dehydrogenase complex and the acetyl-CoA then fully oxidized to CO_2 in the TCA cycle.

1. Amino acids converted to pyruvate
   Alanine, serine, glycine, threonine, cysteine, tryptophan
2. Amino acids converted to oxaloacetate
   Aspartate, asparagine
3. Amino acids converted to α-ketoglutarate
   Glutamate, glutamine, proline, histidine, arginine
4. Amino acids converted to fumarate
   Phenylalanine, tyrosine
5. Amino acids converted to succinyl-CoA
   Methionine, isoleucine, valine
6. Amino acids converted to acetyl-CoA
   Leucine, isoleucine, lysine, phenylalanine, tyrosine, tryptophan, threonine

because it is a common route for the generation of PEP from oxalacetate to be used for gluconeogenesis. Alternatively, in muscle, PEP can be converted to pyruvate that can be decarboxylated to acetyl-CoA for subsequent oxidation to CO_2 in the TCA cycle.

The regulation of anaplerosis and cataplerosis depends upon the metabolic and physiologic state and the specific tissue/organ involved. For example, during starvation, cataplerosis via phosphoenolpyruvate to support gluconeogenesis may be regulatory in the liver, whereas in the kidney anaplerosis via uptake of glutamine may be regulatory. Anaplerotic and cataplerotic intermediates entering and exiting the TCA cycle are shown in Fig. 1. A detailed and elegant analysis of amino acid metabolism can be found in a review by Jungas *et al.* (3).

Physiological Role of Cataplerosis and Anaplerosis in Metabolism of Glutamine in Human Kidney

The interplay between anaplerotic and cataplerotic reactions in humans was demonstrated by renal metabolism during total, prolonged starvation (4). Arteriovenous concentration differences of metabolites across the kidneys coupled with urinary nitrogen losses showed that the kidney extracted glutamine and produced urinary ammonium (5). Concurrently, the kidney released glucose into the blood. It was initially recognized that renal ammoniagenesis was related to ketonuria during prolonged starvation when there is an increase in ketogenesis (6). However, it was not generally appreciated that the entry (anaplerosis) and removal (cataplerosis) of intermediates into and out of the TCA cycle as related to renal ammoniagenesis and gluconeogenesis had to be balanced. This fundamental principle is poorly understood and is the foundation of this paper.

During prolonged starvation glutamine is transported from muscle to the kidney where the amino and amide groups are used for ammonia formation. The ammonia released from the renal cells serves to titrate the acidity of the tubular urine created by the disassociation of organic acids, primarily β-hydroxybutyric and acetoacetic acids. For ammonia generation to continue, glutamine undergoes anaplerotic reactions to form α-ketoglutarate that enters the TCA cycle and is sequentially converted to malate that leaves the mitochondria. Malate is oxidized in the cytosol to oxalacetate that is subsequently converted to PEP and then to glucose. Thus, anaplerotic and cataplerotic reactions are essential and balanced during renal ammoniagenesis and gluconeogenesis.

The heightened ketonuria that occurs with ketonemia is related to the need for the kidney to generate glucose during total starvation when renal gluconeogenesis accounts for about 50% of the net glucose synthesis (4, 7). Thus, renal ammoniagenesis and gluconeogenesis are tightly interlocked and dependent upon balanced anaplerotic reactions to replenish the α-ketoglutarate in the TCA cycle and cataplerotic reactions to drain remnant 4-carbon metabolic intermediates from the cycle to synthesize glucose (7). In addition, there is a metabolic bonus when the kidneys excrete urinary ammonium during starvation. The caloric value of protein is greater when amino acid nitrogen is lost in the urine as ammonium rather than urea because it requires four molecules of ATP to generate a molecule of urea via the urea cycle. In addition, energy is required for the synthesis of creatine and uric acid.

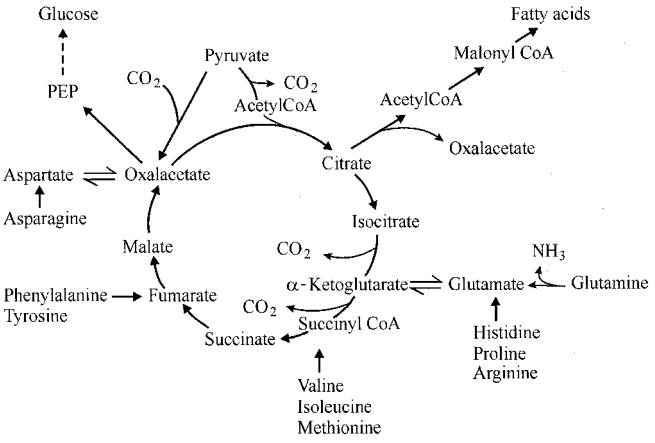

FIG. 1. **Anaplerosis and cataplerosis in the TCA cycle.** The TCA cycle is presented with the major anaplerotic and cataplerotic reactions illustrated. These include the net entry of amino acids into the cycle and the generation of oxaloacetate from pyruvate via pyruvate carboxylase. The cataplerotic reactions in the figure illustrate the linkage of this process to both gluconeogenesis and lipogenesis.

Physiological Role of Anaplerosis and Cataplerosis in Amino Acid Metabolism in Human Skeletal Muscle

Despite the relatively slow rate of turnover of skeletal muscle protein, it represents the largest reservoir of amino acids because of its large mass. Following an overnight fast, there is a net release of amino acids from skeletal muscle; however, the amino acids released do not reflect the amino acid composition of the skeletal muscle proteins (3, 8). This suggests that there is local metabolism and interconversion of amino acids in the muscle. Specifically, alanine and glutamine represent a disproportionately larger fraction of amino acids released by the skeletal muscle when compared with the amino acid composition of skeletal muscle proteins. The relative proportion of these amino acids released by muscle also changes with the metabolic status, such as prolonged starvation or diabetes, or in response to administration of insulin or glucagon (3, 8–11). These data suggest that a local metabolism of amino acids occurs in the skeletal muscle that results in the *de novo* synthesis of certain non-essential amino acids, primarily alanine and glutamine. Arteriovenous concentration differences across skeleton muscles show net uptake and/or release of lactate, ammonia, alanine, glutamine, and glutamate at rest and during exercise (11). In addition, during exercise there is an increase in the TCA cycle intermediates; however, the increase in concentration is not equal in all the TCA intermediates (Table II).

During caloric restriction, amino acids also provide a source of energy. Amino acids yield part of their energy during oxidative deamination, but their carbon skeleton must undergo subsequent catabolism to be fully metabolized. Although most amino acids enter the TCA cycle as 4- or 5-carbon compounds, only acetyl-CoA produced from their catabolism can be fully

TABLE II
Intramuscular concentrations of individual TCA cycle intermediates at rest and during exercise
Values are mean ± S.E. in mmol/kg, dry weight; $n = 6$. Credit for this table should be given to Gibala *et al*. (11).

	Rest	5 min	10 min
Citrate	0.362 ± 0.047	0.658 ± 0.077[a]	0.631 ± 0.052[a]
Isocitrate	0.085 ± 0.013	0.194 ± 0.022[a]	0.200 ± 0.022[a]
α-Ketoglutarate	0.050 ± 0.004	0.036 ± 0.005[a]	0.038 ± 0.005[a]
Succinate	0.368 ± 0.076	0.567 ± 0.115	0.609 ± 0.118
Fumarate	0.087 ± 0.006	0.198 ± 0.029[a]	0.195 ± 0.032[a]
Malate	0.365 ± 0.037	1.163 ± 0.203[a]	1.182 ± 0.160[a]
Oxalacetate	0.012 ± 0.003	0.030 ± 0.005[a]	0.027 ± 0.006[a]

[a] $p \leq 0.05$ *versus* rest.

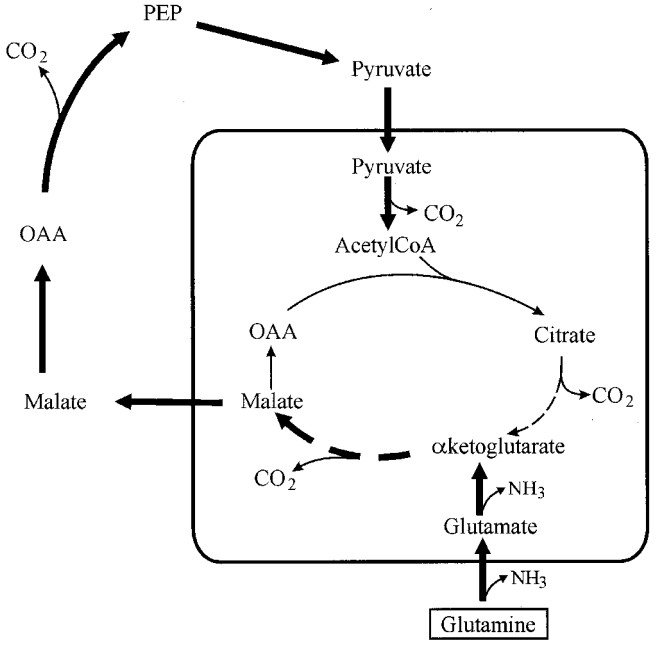

FIG. 2. **The role of anaplerosis and cataplerosis in the metabolism of glutamine by the small intestine.** The small intestine metabolizes glutamine for energy via the TCA cycle. The entry of glutamine into the cycle (anaplerosis) is balanced by its removal (cataplerosis) as malate. The malate is subsequently converted to oxaloacetate (*OAA*) and then to PEP via PEPCK. The PEP can then be converted to pyruvate by pyruvate kinase for entry into the TCA cycle as acetyl-CoA. In addition, pyruvate may be transaminated to alanine.

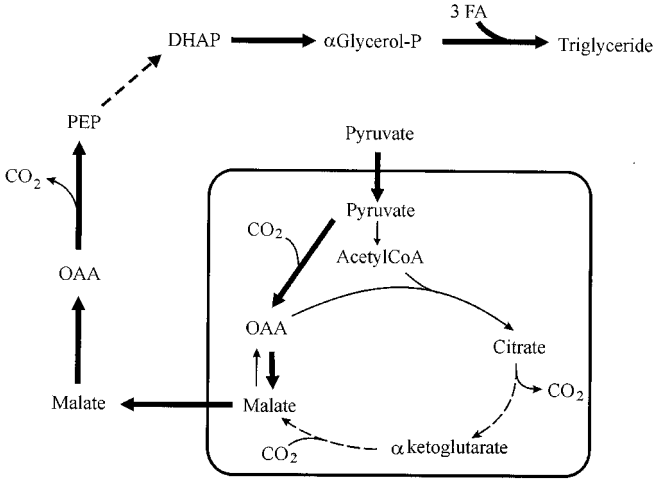

FIG. 3. **Linkage of anaplerosis, cataplerosis, and glyceroneogenesis in adipose tissue.** The pathway of glyceroneogenesis from pyruvate is illustrated to stress the balance of anaplerosis (the entry of oxaloacetate (*OAA*) synthesized from pyruvate via pyruvate carboxylase) and cataplerosis (the removal of intermediates to support the synthesis of glyceride-glycerol). *FA*, fatty acid; *DHAP*, dihydroxyacetone phosphate.

oxidized in the cycle. Recent data from studies in humans have shown that only a small increase in the concentration of TCA intermediates (mostly 4-carbon) occurs during fasting or exercise, thus confirming the concept that only small changes in the amounts of these intermediates are required to adapt to the need for energy (12, 13).

The removal (cataplerosis) of TCA cycle anions generated from the entry of amino acids occurs via the action of PEPCK, glutamate dehydrogenase, or aspartate aminotransferase. Alanine and glutamine are synthesized from other amino acids and released into the circulation. Alanine is generated by the transamination of pyruvate via alanine aminotransferase. The possible sources of pyruvate are glucose and lactate, or PEP via PEPCK, using as a substrate the oxalacetate generated in the TCA cycle (cataplerosis) (13).

The carbon skeleton for the synthesis of glutamine can be generated from the TCA cycle intermediates formed by the catabolism of gluconeogenic amino acids such as aspartate and asparagine (14). These amino acids are capable of generating intermediates that can be converted by forward flow of the TCA cycle to the α-ketoglutarate required for glutamate synthesis. Transamination of α-ketoglutarate, using the branched-chain amino acids as the source of the amino groups, accounts for the synthesis of glutamate, which is then converted to glutamine by glutamine synthase using ammonia generated in muscle by the purine nucleotide cycle. In light of the concept of balanced anaplerosis and cataplerosis, branched-chain amino acid metabolism in muscle needs to be studied further.

Physiological Role of Anaplerosis and Cataplerosis in Metabolism of Glutamine by Human Small Intestine

Another paradigm for the metabolic roles of anaplerosis and cataplerosis is the oxidation of glutamine to CO_2 in the small intestine. In this case, the α-ketoglutarate formed from glutamine is converted to malate by the TCA cycle; the malate leaves the mitochondria and is oxidized in the cytosol to oxalacetate by NAD:malate dehydrogenase. The oxalacetate is decarboxylated to PEP by PEPCK, and the PEP is converted to pyruvate by pyruvate kinase. The pyruvate re-enters the mitochondria where it is decarboxylated to acetyl-CoA by the pyruvate dehydrogenase complex; the acetyl-CoA is then oxidized by the TCA cycle. Alternately, a fraction of the pyruvate may be transaminated to alanine in the cytosol (Fig. 2).

Recent research on the metabolism of glutamine in human adults and infants illustrates the critical role of anaplerosis and cataplerosis in amino acid metabolism in the gut. In normal healthy adults, almost 74% of the enterally administered glutamine was extracted by the splanchnic compartment during the first pass (15), whereas 70–80% of enterally administered [^{13}C]glutamine tracer was found in respiratory CO_2 (16). Other data from studies of newborn infants also show that enterally administered glutamine is rapidly metabolized in the

gut as an energy source for the enterocytes, resulting in increased urea production (17). The majority of the glutamine is metabolized by the small intestine and not the liver, because there is no observed dilution of parenterally administered [^{15}N]glutamine. This suggests that the carbon skeleton of dietary glutamine is oxidized by the small intestine (not the liver) as a source of energy.

The metabolism of glutamine by the small intestine illustrates the importance of both anaplerosis and cataplerosis in the metabolism of the 5-carbon intermediates formed from the catabolism of the carbon skeletons of amino acids. For the carbon skeleton of glutamine to be metabolized to CO_2 in the TCA cycle, the α-ketoglutarate formed from glutamate (originally from the deamination of glutamine) must be converted to acetyl-CoA for full oxidation. As shown in Fig. 2, the removal of carbon from the TCA cycle involves the cataplerotic activity of PEPCK in the small intestine, which synthesizes PEP from the oxalacetate generated from the oxidation of α-ketoglutarate.

Role of Cataplerosis in Synthesis of Triglyceride (Glyceroneogenesis) in Adipose Tissue during Fasting

The synthesis of triglyceride in adipose tissue during starvation is another example of cataplerosis linked to a biosynthetic pathway. There is a net breakdown of triglyceride in adipose tissue during fasting (lipolysis) that is stimulated by cAMP and inhibited by insulin. During starvation when the rates of lipolysis are highest, a major fraction (up to 30%) of the free fatty acids generated from triglyceride breakdown is re-esterified back to triglyceride in adipose tissue (18–21). This process requires a source of 3-glycerol phosphate, which is generally supplied by glucose via glycolysis. However, during prolonged starvation glucose utilization by adipose tissue is curtailed to spare glucose as a fuel for the brain and red blood cells. An alternative source of 3-glycerol phosphate is provided by *glyceroneogenesis,* an abbreviated version of gluconeogenesis, which provides the glyceride glycerol in triglyceride in adipose tissue (Fig. 3) (22, 23). Rats fed a high protein, carbohydrate-free diet synthesize up to 80% of their glyceride glycerol in white adipose tissue by glyceroneogenesis (24).

Glyceroneogenesis is also an important pathway for the synthesis of hepatic triglyceride during fasting. It has been estimated that ~50% of the fatty acids taken up by the liver during fasting is converted to triglyceride and released as very low density lipoproteins (7). Glyceroneogenesis is a major pathway for the synthesis of the 3-glycerol phosphate that is required to support triglyceride synthesis in the liver. Studies by Botion *et al.* (24) have established that glyceroneogenesis in the livers of rats fed a high protein, carbohydrate-free diet contributes 80% of the glyceride glycerol in circulating very low density lipoproteins. In humans who have fasted for 18 h, 25–60% of the glyceride glycerol is derived from glyceroneogenesis and only 5% from plasma glycerol (25).

Conclusions

The TCA cycle is delicately balanced between the inflow and output of intermediates for various metabolic processes. The widely held view of the TCA cycle as a "metabolic furnace" needs modification in light of information supporting its role in biosynthesis. The cycle acts more as a traffic circle on a busy highway in which the flow of cars into the circle must be balanced by the flow out or the entire traffic pattern will be interrupted with disastrous consequences. In this essay we have reviewed several metabolic situations in which the two key processes, anaplerosis and cataplerosis, work together to ensure the appropriate balance of carbon flow into and out of the TCA cycle. The beauty of this fundamental biological mechanism is undeniable in its simplicity and ponderous in its complexity. It is perhaps as Edmund said in King Lear:

> Thou hast spoken right, 'tis true.
> The wheel is come full circle.

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