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See corresponding article on page 2235.

What Constitutes a Gluconeogenic Precursor?

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ABSTRACT

A gluconeogenic precursor is a biochemical compound acted on by a gluconeogenic pathway enabling the net synthesis of glucose. Recognized gluconeogenic precursors in fasting placental mammals include glycerol, lactate/pyruvate, certain amino acids, and odd-chain length fatty acids. Each of these precursors is capable of contributing net amounts of carbon to glucose synthesis via the tricarboxylic acid cycle (TCA cycle) because they are anaplerotic, that is, they are able to increase the pools of TCA cycle intermediates by the contribution of more carbon than is lost via carbon dioxide. The net synthesis of glucose from even-chain length fatty acids (ECFAs) in fasting placental mammals, via the TCA cycle alone, is not possible because equal amounts of carbon are lost via carbon dioxide as is contributed from fatty acid oxidation via acetyl-CoA. Therefore, ECFAs do not meet the criteria to be recognized as a gluconeogenic precursor via the TCA cycle alone. ECFAs are gluconeogenic precursors in organisms with a functioning glyoxylate cycle, which enables the net contribution of carbon to the intermediates of the TCA cycle from ECFAs and the net synthesis of glucose. The net conversion of ECFAs to glucose in fasting placental mammals via C3 metabolism of acetone may be a competent though inefficient metabolic path by which ECFA could be considered a gluconeogenic precursor. Defining a substrate as a gluconeogenic precursor requires careful articulation of the definition, organism, and physiologic conditions under consideration. *J Nutr* 2020;150:2239–2241.

Keywords: acetone, anaplerotic reactions, fatty acids, gluconeogenesis, gluconeogenic precursors, glyoxylate cycle, tricarboxylic acid cycle, precursor

Introduction

What constitutes a gluconeogenic precursor? To adequately discuss and answer this question, it is necessary to carefully define terms, state the organism, and physiologic conditions. A precursor is a biochemical compound preceding another in a metabolic pathway. A gluconeogenic precursor is a biochemical compound acted on by a gluconeogenic pathway enabling the net synthesis of glucose (1). A substrate may meet the definition of precursor but fail to meet the definition of gluconeogenic precursor if it does not enable the net synthesis of glucose, as will be discussed below. Metabolic capabilities vary widely among organisms, for the sake of discussion we first consider the case of a placental mammal, in the fasted state, actively synthesizing glucose.

Applying these definitions and conditions, recognized gluconeogenic precursors include glycerol, lactate/pyruvate, odd-chain length fatty acids, and gluconeogenic amino acids (alanine, arginine, asparagine, aspartic acid, cysteine, glutamic acid, glutamine, glycine, histidine, methionine, proline, serine, valine, phenylalanine, isoleucine, threonine, tryptophan, and tyrosine). Each of these precursors is capable of contributing net amounts of carbon to glucose synthesis via the tricarboxylic acid cycle (TCA cycle) because they are anaplerotic – that is, they are able to increase the pools of TCA cycle intermediates by the contribution of more carbon than is lost via carbon dioxide (2). Glycerol enters the TCA cycle via pyruvate (or may enter upstream via dihydroxyacetone phosphate), gluconeogenic amino acids via pyruvate, oxaloacetate, fumarate, succinyl-CoA, and α-ketoglutarate, and odd-chain length fatty acids via propionyl-CoA metabolism to succinyl-CoA. On this basis, 2 forms of "fat," triacylglycerols (via glycerol) and odd-chain length fatty acids are considered gluconeogenic precursors via the TCA cycle alone. These are gluconeogenic precursors because each can provide net carbon flux into glucose. In a word, each of these carbon sources is "anaplerotic," whereas acetyl-CoA is not anaplerotic (2).

Now consider the case of glucose synthesis from even-chain fatty acids (ECFAs) in a fasting placental mammal. Although ECFAs meet the definition of a precursor, when they are assessed via the canonical TCA cycle pathway alone, ECFAs do not meet the definition of a gluconeogenic precursor because they do not enable the net synthesis of glucose. Entry of carbon from ECFAs into the TCA cycle is via acetyl-CoA's condensation with oxaloacetate, catalyzed by citrate synthase,

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Abbreviations used: ECFA, even-chain fatty acid; ISL, isocitrate lyase; MS, malate synthase; TCA cycle, tricarboxylic acid cycle.

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TABLE 1 Substrate carbon stoichiometry of the 8 reactions of the TCA cycle and their summation when carbon enters the TCA cycle via acetyl-CoA from ECFAs (without entry of carbon from anaplerotic sources) demonstrating no net availability of carbon for glucose biosynthesis

Enzyme	Carbon reactants		Total substrate carbon		Carbon products		Total product carbon
Citrate synthase	Oxaloacetate	**Acetyl-CoA**	6	↔	Citrate		6
Aconitase	Citrate		6	↔	Isocitrate		6
Isocitrate dehydrogenase	Isocitrate		6	↔	α-Ketoglutarate	CO_2	6
α-Ketoglutarate dehydrogenase	α-Ketoglutarate		5	↔	Succinyl-CoA	CO_2	5
Succinyl-CoA synthetase	Succinyl-CoA		4	↔	Succinate		4
Succinate dehydrogenase	Succinate		4	↔	Fumarate		4
Fumarase	Fumarate		4	↔	Malate		4
Malate dehydrogenase	Malate		4	↔	Oxaloacetate		4
Summed, net reaction		Acetyl-CoA	2	↔		**2 CO_2**	2

Bolded text highlights entry of carbon as Acetyl-CoA and its exit as CO_2. ECFA, even-chain fatty acid; TCA cycle, tricarboxylic acid cycle.

which occurs prior to the decarboxylation reactions in the TCA cycle. The stoichiometry of the TCA cycle is outlined in Table 1, emphasizing its role as a catalytic acetate oxidation cycle. After each of the 8 enzymes have functioned 1 time, all of the carbon intermediates of the cycle return to their starting point. The only net change is that acetate has been combusted and energy (in GTP and reducing equivalents) has been generated.

If carbon exits the cycle (e.g., for gluconeogenesis) the collective pool size of the intermediates is diminished, which is not sustainable. The carbon that exits must be replaced via anaplerotic reactions which result in net carbon addition from gluconeogenic precursors. Reworded, citrate synthase is not an anaplerotic reaction, it does not replenish the pool size of TCA cycle carbon and it does not provide net gluconeogenic carbon. This stoichiometry (Table 1) doesn't allow the contribution of net carbon to glucose, even though radiolabeled carbon atoms originating in ECFAs may be incorporated into glucose due to the well-understood labeling pattern described by Weinman et al. (3) and illustrated by Green (4). In contrast, Table 2 outlines anaplerosis from α-ketoglutarate which channels carbon from glutamate, glutamine, arginine, histidine, and proline, and can contribute net carbon for gluconeogenesis.

If the determination of a gluconeogenic precursor was made only on the basis of contribution of carbon atoms and not on the net contribution of carbon to glucose, then along with ECFA, one would also have to consider carbon dioxide a gluconeogenic precursor because, if labeled, it too will label the glucose pool (5). It would be erroneous to conclude that animals (like plants) can fix carbon dioxide for the net reductive biosynthesis of glucose. In accord, we contend that neither carbon dioxide nor ECFAs meet the definition of a gluconeogenic precursor. Readers are referred to the isotopomer analyses of Brunengraber and coworkers to understand why caution and care are needed when interpreting isotope data for measuring net gluconeogenesis (6).

Conditions Under Which ECFAs Meet or May Meet the Definition of a Gluconeogenic Precursor

Bacteria, fungi, single-celled eukaryotic organisms, plants, and nematodes are recognized as capable of the net conversion of ECFAs to glucose (7–9). The presence of a functioning glyoxylate cycle [malate synthase (MS) and isocitrate lyase

TABLE 2 Substrate carbon stoichiometry of the 8 reactions of the TCA cycle and their summation with carbon entry via acetyl Co-A from ECFA and entry of carbon from the anaplerotic substrate α-ketoglutarate, demonstrating the net synthesis of 1 oxaloacetate which is available for glucose biosynthesis

Enzyme	Carbon reactants		Total substrate carbon		Carbon products		Total product carbon
Citrate synthase	Oxaloacetate	Acetyl-CoA	6	↔	Citrate		6
Aconitase	Citrate		6	↔	Isocitrate		6
Isocitrate dehydrogenase	Isocitrate		6	↔	α-Ketoglutarate	CO_2	6
α-Ketoglutarate dehydrogenase	α-Ketoglutarate **→ α-Ketoglutarate**		10	↔	2 Succinyl-CoA	2 CO_2	10
Succinyl-CoA synthetase	2 Succinyl-CoA		8	↔	2 Succinate		8
Succinate dehydrogenase	2 Succinate		8	↔	2 Fumarate		8
Fumarase	2 Fumarate		8	↔	2 Malate		8
Malate dehydrogenase	2 Malate		8	↔	2 Oxaloacetate		8
Summed, net reaction	α-Ketoglutarate	Acetyl-CoA	7	↔	**→ Oxaloacetate**	3 CO_2	7
Contrast with the result of the summed, net reaction when carbon enters the TCA cycle from ECFAs only via acetyl-CoA, from Table 1:							
Summed, net reaction		Acetyl-CoA	2	↔		2 CO_2	2

Bolded text highlights entry of anaplerotic carbon as α-ketoglutarate and the resulting net production of oxaloacetate. ECFA, even-chain fatty acid; TCA cycle, tricarboxylic acid cycle

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(ISL)] enables these organisms to contribute net amounts of carbon to the intermediates of the TCA cycle and convert ECFAs into net amounts of glucose. Although there have been reports of either MS or ISL activity, or both MS and ISL activity in higher animals (birds, reptiles, placental mammals including hibernating bears) evidence of a functioning glyoxylate cycle remains controversial (7–10).

A cross-species genomic analysis by Kondrashov et al. (9) did not find evidence of functional *MS* and *ISL* genes present together in higher animals suggesting that the genetic coding to support a functioning glyoxylate cycle may not be present. Similarly, a metabolic pathway analysis of the question under consideration by de Figueiredo et al. (7) did not find evidence of a competent glyoxylate cycle. Although not definitive, these studies support the position that higher animals do not have the genetic coding nor the metabolic pathways for the net conversion of ECFAs to glucose via a functioning glyoxylate cycle.

Over the years there have been reports of the conversion or potential conversion of ECFAs to glucose via acetone (11–19) and calls to consider ECFAs gluconeogenic precursors via this metabolic pathway (20, 21). Kaleta et al. (8) considers in silico stoichiometrically feasible metabolic routes in detail, finding a number of competent though limited throughput paths (energetically and because of high requirements for reducing equivalents) from ECFAs to glucose via acetone. Although the net conversion of ECFAs to glucose in higher animals via C3 metabolism of acetone may be a competent metabolic path, the quantitative importance under physiological conditions appears to be limited to 11% or less of synthesized glucose in fasting humans (14, 17, 18) and rats (15) compared with >90% of net synthesized glucose in humans being derived from lactate/pyruvate, glutamine, alanine, and glycerol (22, 23).

Conclusions

What constitutes a gluconeogenic precursor in fasted placental mammals? When a gluconeogenic precursor is defined as enabling the net synthesis of glucose (anaplerotic), then glycerol, pyruvate/lactate, gluconeogenic amino acids, and odd-chain length fatty acids meet the definition of a gluconeogenic precursor via the TCA cycle alone. While some carbon atoms of ECFAs end up in glucose, ECFAs are not net contributors of carbon to glucose synthesis via the TCA cycle alone in fasted placental mammals. Therefore, ECFAs are not gluconeogenic precursors in fasted placental mammals via the TCA cycle alone. That is not to say that ECFAs do not play an important role in gluconeogenesis – they do by producing ATP/GTP and reducing equivalents.

ECFAs are gluconeogenic precursors by this definition in organisms demonstrated to have a functioning glyoxylate cycle, which enables the net conversion of ECFAs to glucose. The net conversion of ECFAs to glucose via acetone in fasted placental mammals appears possible and may achieve consensus with additional data. This recognition would support ECFAs as a gluconeogenic precursor under these conditions by this metabolic path. Though the conversion of ECFAs to glucose via acetone may be inefficient and limited, it could be physiologically important in starvation.

In biology, definitive statements regarding metabolism require careful articulation of the definitions, organisms, and physiologic conditions under consideration. Even then definitive statements are made in peril of the next reported data set. To be clear, no new data are presented herein. When new data do avail, it is critical to clearly state the parameters associated with novel inferences and allow the new data to foster reevaluation and a new interpretation or hypothesis.

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