Understanding the interrelationship between the synthesis of urea and gluconeogenesis by formulating an overall balanced equation

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Ipata PL, Pesi R. Understanding the interrelationship between the synthesis of urea and gluconeogenesis by formulating an overall balanced equation. Adv Physiol Educ 41: 286–290, 2017; doi: 10.1152/advan.00180.2016.—It is well known that a strong metabolic interrelationship exists between ureagenesis and gluconeogenesis. In this paper, we present a detailed, overall equation, describing a possible metabolic link between ureagenesis and gluconeogenesis. We adopted a guided approach in which we strongly suggest that students, when faced with the problem of obtaining the overall equation of a metabolic pathway, carefully account for all atoms and charges of the single reactions, as well as the cellular localizations of the substrates, and the related transport systems. If this suggestion is always taken into account, a balanced, overall equation of a metabolic pathway will be obtained, which strongly facilitates the discussion of its physiological role. Unfortunately, textbooks often report unbalanced overall equations of metabolic pathways, including ureagenesis and gluconeogenesis. Most likely the reason is that metabolism and enzymology have been neglected for about three decades, owing to the remarkable advances of molecular biology and molecular genetics. In this paper, we strongly suggest that students, when faced with the problem of obtaining the overall reaction of a metabolic pathway, carefully control if the single reactions are properly balanced for atoms and charges. Following this suggestion, we were able to obtain an overall equation describing the metabolic interrelationship between ureagenesis and gluconeogenesis, in which urea and glucose are the final products. The aim is to better rationalize this topic and to convince students and teachers that metabolism is an important and rewarding chapter of human physiology.

overall reaction of ureagenesis; overall reaction of gluconeogenesis; interaction between ureagenesis and gluconeogenesis; importance of balanced equations

Introduction: the Equations of Urea Synthesis and of Gluconeogenesis

The synthesis of urea, often referred to as “ureagenesis” or “urea cycle” was discovered by Krebs and Henseleit in 1932 (12). It occurs predominantly in the liver (3, 6, 11, 14, 15, 28). Its physiological importance in handling the toxic ammonium ions discharged during catabolism of amino acids is satisfactorily discussed in many texts. The overall equation of ureagenesis (Table 1) is:

\[
\text{NH}_4^+ \text{mit} + \text{HCO}_3^- \text{mit} + 3 \text{ATP}^4- + \text{Asp}^1-_{\text{cyt}} + \text{H}_2\text{O} \rightarrow \text{urea}_{\text{cyt}} + 2 \text{ADP}^3- + 2 \text{P}_i^2- + \text{AMP}^2- + \text{PP}_i^4- + \text{fumarate}^2-_{\text{cyt}} + 5 \text{H}^+ (1)
\]

where Asp is aspartate, mit is mitochondrial, and cyt is cytosolic.

Gluconeogenesis is the pathway by which glucose is generated, starting from amino acids or lactate converted to pyruvate. The overall equation (Table 2) is:

\[
2 \text{ pyruvate}^1-_{\text{cyt}} + 4 \text{ ATP}^4- + 2 \text{ GTP}^4- + 2 \text{ NADH} + 6 \text{ H}_2\text{O} \rightarrow \text{glucose}^1-_{\text{cyt}} + 2 \text{ NAD}^1+ + 4 \text{ ADP}^3- + 2 \text{ GDP}^3- + 6 \text{ P}_i^2- + 2 \text{ H}^+ (2)
\]

At first glance, both equations may appear too complicated to be memorized and discussed. However, taking into account the familiar formulas of urea (H$_2$N-CO-NH$_2$) and fumarate ("OOO-CH = CH-COO"), the two final products of ureagenesis (Eq. 1) can be “read” as follows: 1) one of the two nitrogen atoms of urea derives from NH$_4^+$ and the other from aspartate, and the carbon atom from HCO$_3^-$; 2) the (4C)carbon skeleton of aspartate generates the (4C)fumarate molecule. Equation 2 shows that one glucose, a (6C)molecule, is synthesized from two (3C)molecules of pyruvate. Other noncarbohydrate precursors of glucose enter the gluconeogenetic pathway at oxaloacetate and dihydroxyacetone-phosphate (Table 2). Lactate, which accumulates during periods of intense exercise in muscle cytosol, is transformed into pyruvate by lactate dehydrogenase:

\[
\text{lactate}^1-_{\text{cyt}} + \text{NAD}^+ \rightarrow \text{pyruvate}^1-_{\text{cyt}} + \text{NADH} + \text{H}^+
\]

and is converted to glucose by gluconeogenesis. Animals do not convert fatty acids into glucose, however they can convert glycerol, produced by the hydrolysis of triacylglycerol, into dihydroxyacetone-phosphate, via the actions of glycerol kinase and glycerol phosphate dehydrogenase:

\[
\text{glycerol}^1-_{\text{cyt}} + \text{ATP}^4- \rightarrow \text{ADP}^3- + \text{glycerol-3-phosphate}^2-_{\text{cyt}} + \text{H}^+ \text{ (glycerol kinase)}
\]

\[
\text{glycerol-3-phosphate}^2-_{\text{cyt}} + \text{NAD}^+ \rightarrow \text{dihydroxyacetone-phosphate}^2-_{\text{cyt}} + \text{NADH} + \text{H}^+ \text{ (glycerol phosphate dehydrogenase)}
\]

Equations 1 and 2 also show that both require the hydrolysis of “high-energy” nucleoside triphosphates to render the two processes exergonic. Students are referred to Tables 1 and 2, reporting the enzymatic and transport steps of the two meta-
Table 1. The reactions, enzymes, transporters, and overall equation of the urea synthesis

<table>
<thead>
<tr>
<th>Reactions</th>
<th>Enzymes</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 NH₄⁺ + HCO₃⁻ + 2 ATP⁺ → carbamoyl phosphate + 2 ADP⁻ + P²⁻ + 2 H⁺</td>
<td>Carbamoyl phosphate synthetase 1</td>
</tr>
<tr>
<td>2 Ornithine + 2 ATP⁺ + Asp⁻ + H₂O → argininosuccinate + AMP⁻ + PPitamin</td>
<td>Ornithine-transcarbamylase</td>
</tr>
<tr>
<td>3 Citrulline + H₂O → ornithine + PEP</td>
<td>Citrulline-ornithine transporter</td>
</tr>
<tr>
<td>4 Argininosuccinate + ATP⁺ + Asp⁻ + H₂O → arginine + fumarate</td>
<td>Argininosuccinate synthetase</td>
</tr>
<tr>
<td>5 Arginine + PEP + ornithine + ATP⁺ + H₂O → urea + H⁺</td>
<td>Arginase</td>
</tr>
<tr>
<td>6 Ornithine + H₂O → ornithine + PEP</td>
<td>Citrulline-ornithine transporter</td>
</tr>
<tr>
<td>Overall equation: NH₄⁺ + HCO₃⁻ + 3 ATP⁺ + Asp⁻ + H₂O → urea + 2 ADP⁻ + AMP⁻ + PPitamin + 2 P²⁻ + fumarate + 5 H⁺</td>
<td></td>
</tr>
</tbody>
</table>

The synthesis of urea is the result of the cross talk between mitochondria and the cytosolic compartment: two mitochondrial reactions (reactions 1 and 2), three cytosolic reactions (reactions 4, 5, and 6), and the transport of two intermediates from mitochondria to cytosol and vice versa (steps 3 and 6).

The Synthesis of Phosphoenolpyruvate from the Cytosolic Fumarate Generated by Ureagenesis

As shown in Eq. 1 and in Table 1 (see also Fig. 1), ureagenesis produces fumarate, a dicarboxylic acid, in addition to urea. In the cytosol, fumarate is acted upon by the successive action of three cytosolic enzymes: fumarase (22), malate dehydrogenase (20, 21), and cytosolic phosphoenolpyruvate (PEP) carboxy kinase (PEPCK-C) (19).

fumarate → malate → (cytosolic fumarase)
malate + NAD⁺ → OAA + NADH + H⁺ (cytosolic malate dehydrogenase)
OAA + GTP → PEP + GDP + CO₂ (PEPCK-C)
where OAA is oxaloacetate.

The overall equation of these three reactions is:

Table 2. The reactions, enzymes, transporters, and overall equation of gluconeogenesis

<table>
<thead>
<tr>
<th>Reactions</th>
<th>Enzymes</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 2 pyruvate → 2 pyruvate</td>
<td>Mitochondrial pyruvate transporter</td>
</tr>
<tr>
<td>2 pyruvate → CO₂ + 2 ATP⁺ + 2 H₂O → oxaloacetate + 2 ADP⁻ + 2 P²⁻ + 4 H⁺</td>
<td>Mitochondrial pyruvate carboxylase</td>
</tr>
<tr>
<td>3 oxaloacetate + NAD⁺ → 2 malate + NAD⁺</td>
<td>Mitochondrial malate dehydrogenase</td>
</tr>
<tr>
<td>4 malate → 2 malate</td>
<td>Mitochondrial malate transporter</td>
</tr>
<tr>
<td>5 malate + NAD⁺ → oxaloacetate + NAD⁺ + 2 H⁺</td>
<td>Malate dehydrogenase</td>
</tr>
<tr>
<td>6 oxaloacetate + 2 GTP → PEP + 2 CO₂ + 2 GDP⁻</td>
<td>PEP carboxy kinase</td>
</tr>
<tr>
<td>7 PEP + 2 CO₂ + NAD⁺ → 2 phosphoglycerate</td>
<td>Enolase</td>
</tr>
<tr>
<td>8 phosphoglycerate → 2-phosphoglycerate</td>
<td>Phosphoglycerate mutase</td>
</tr>
<tr>
<td>9 3-phosphoglycerate + 2 ATP⁺ → 2,3-bisphosphoglycerate + 2 ADP⁻</td>
<td>Phosphoglycerate kinase</td>
</tr>
<tr>
<td>10 2,3-bisphosphoglycerate + 2 NAD⁺ + 2 H⁺ → glyceraldehyde-3-phosphate + 2 NAD⁺ + 2 P²⁻</td>
<td>3-Phosphoglycerate dehydrogenase</td>
</tr>
<tr>
<td>11 glyceraldehyde-3-phosphate + dihydroxyacetone-phosphate</td>
<td>Triose phosphate mutase</td>
</tr>
<tr>
<td>12 glyceraldehyde-3-phosphate + dihydroxyacetone-phosphate → fructose-1,6-bisphosphate</td>
<td>Aldolase</td>
</tr>
<tr>
<td>13 fructose-1,6-bisphosphate + H₂O → fructose-6-phosphate + P²⁻</td>
<td>Fructose-1,6-bisphosphatase</td>
</tr>
<tr>
<td>14 fructose-6-phosphate → glucose-6-phosphate</td>
<td>Phosphoglucomutase</td>
</tr>
<tr>
<td>15 glucose-6-phosphate + H₂O → glucose + P²⁻</td>
<td>Glucose-6-phosphatase</td>
</tr>
</tbody>
</table>

Overall equation: 2 pyruvate + 4 ATP⁺ + 2 GTP⁺ + 2 NAD⁺ + 6 H₂O → glucose + 2 NAD⁺ + 4 ADP⁻ + 2 GDP⁻ + 6 P²⁻ + 2 H⁺

Two (3C)molecules are needed to synthesize one (6C)glucose molecule. Therefore, reactions 1–10 are multiplied by 2. The single reactions are catalyzed by cytosolic enzyme proteins. However, steps 1–4 require the intervention of 2 mitochondrial transporters and 2 mitochondrial enzyme proteins. The overall equation of gluconeogenesis starting from 2 cytosolic PEP molecules (reactions 7–15) is 2 PEP⁻ + 4 H₂O + 2 ATP⁺ + 2 NAD⁺ + 2 H⁺ → glucose + 2 ADP⁻ + P²⁻ + 2 NAD⁺.
Fig. 1. Metabolic relationship between the ureagenesis and the gluconeogenesis pathways. The figure shows that one turn of the urea cycle generates one cytosolic fumarate and one cytosolic urea. Urea is excreted by urine, whereas fumarate and one mitochondrial pyruvate generate two cytosolic PEPs, which are then converted to glucose by gluconeogenesis. Cytosolic fumarate is converted to cytosolic PEP via cytosolic fumarase and one cytosolic urea. Urea is excreted by urine, whereas fumarate and one mitochondrial pyruvate generate two cytosolic PEPs, which are followed by mitochondrial malate dehydrogenase, malate, and PEPCK-C (19). Other enzymes and transporters are as follows: 1) carbamoyl-phosphate synthetase; 2) ornithine transcarbamylase; 3) cytosolic malate dehydrogenase; 4) argininosuccinate synthetase; 5) argininosuccinase; 6) arginine. The overall reaction of the gluconeogenic pathway starting from two PEPs is reported in Table 1, legend.

By adding this equation to Eq. 1, we can state that one turn of ureagenesis may be coupled to the synthesis of one cytosolic PEP:

\[
\text{NH}_4^{+} + \text{HCO}_3^{-} + 3 \text{ATP}^{4-} + 2 \text{H}_2\text{O} + \text{GTP}^{4-} + \text{Asp}^{1-} + \text{NAD}^{1+} \rightarrow \text{OAA}^{2-} + \text{ADP}^{3-} + \text{AMP}^{2-} + \text{PP}^{4-} + 2 \text{P}^{2-} + \text{GDP}^{3-} + \text{CO}_2 + \text{NADH}_\text{cyt} + 6 \text{H}^{1+} \quad (3)
\]

The other cytosolic PEP molecule, needed to synthesize one glucose molecule by gluconeogenesis (see Table 2 legend) is synthesized starting from mitochondrial pyruvate (Pyr), through one of the two possible pathways shown in Fig. 1. In the first one (the PEPCK-C-mediated gluconeogenesis), pyruvate is acted upon by mitochondrial pyruvate kinase (7, 27), followed by mitochondrial malate dehydrogenase (20), malate transfer into the cytosolic compartment (25), cytosolic malate dehydrogenase (20), and PEPCK-C (19):

\[
\text{Pyr}^{1-} + \text{ATP}^{4-} + \text{HCO}_3^{-} \rightarrow \text{OAA}^{2-} + \text{ADP}^{3-} + \text{P}^{2-} + \text{H}^{1+} \quad \text{(pyruvate carboxylase)}
\]

\[
\text{OAA}^{2-} + \text{NADH}_\text{mit} + \text{H}^{1+} \rightarrow \text{malate}^{2-} + \text{NAD}^{1+} \quad \text{(mitochondrial malate dehydrogenase)}
\]

\[
\text{malate}^{2-} \rightarrow \text{malate}^{2-} \quad \text{(malate 3-oxoglutarate carrier)}
\]

\[
\text{malate}^{2-} + \text{NAD}^{1+} \rightarrow \text{OAA}^{2-} + \text{NADH}_\text{cyt} + \text{H}^{1+} \quad \text{(cytosolic malate dehydrogenase)}
\]

\[
\text{OAA}^{2-} + \text{GTP}^{4-} \rightarrow \text{PEP}^{3-} + \text{GDP}^{3-} + \text{CO}_2 \quad \text{(PEPCK-C)}
\]

By summarizing these five steps, we obtain the following overall reaction for the synthesis of the second PEP molecule, starting with pyruvate:

\[
\text{Pyr}^{1-} + \text{ATP}^{4-} + \text{HCO}_3^{-} + \text{GTP}^{4-} \rightarrow \text{PEP}^{3-} + \text{ADP}^{3-} + \text{P}^{2-} + \text{H}^{1+} + \text{CO}_2 \quad (4)
\]

The second pathway [often named the PEPCK-M (mitochondrial phosphoenolpyruvate carboxy kinase)-mediated PEP gluconeogenesis] (see Fig. 1) is composed of the following three steps:

\[
\text{Pyr}^{1-} + \text{ATP}^{4-} + \text{HCO}_3^{-} \rightarrow \text{OAA}^{2-} + \text{ADP}^{3-} + \text{P}^{2-} + \text{H}^{1+} \quad \text{(pyruvate carboxylase)}
\]

\[
\text{OAA}^{2-} + \text{GTP}^{4-} \rightarrow \text{PEP}^{3-} + \text{GDP}^{3-} + \text{CO}_2 \quad \text{(PEPCK-M)}
\]

\[
\text{PEP}^{3-} \rightarrow \text{PEP}^{3-} \quad \text{(PEP transporter)}
\]

Interestingly, the overall equation of these three reactions is Eq. 4 (see Fig. 1).

We are, therefore, faced with two cytosolic PEP molecules generated by Eq. 3 (the synthesis of one cytosolic PEP via one turn of the ureagenesis coupled with fumarate conversion to PEP), and by Eq. 4 (the synthesis of one cytosolic PEP molecule mediated either by PEPCK-C or by PEPCK-M). It has long been known that a strong interrelationship exists between ureagenesis and gluconeogenesis (18). By summarizing Eqs. 3 and 4 to gluconeogenesis equation, starting from 2
PEP (see Table 2 legend), we obtain, to our knowledge, for the first time, a single balanced equation describing the molecular basis of the interaction between ureagenesis and gluconeogenesis networks, with the production of both urea and glucose.

\[
\begin{align*}
\text{NH}_4^{+} \text{mit} + 2 \text{HCO}_3^{-} \text{mit} + \text{Pyr}^- + 6 \text{ATP}^{4-} + 6 \text{H}_2\text{O} + 2 \text{GTP}^{4-} + \text{Asp}^{1-} \text{cyt} + 2 \text{NAD}^{+} + 2 \text{NADH}_{\text{cyt}} + 2 \text{H}^{+} \rightarrow & \text{urea}_{\text{cyt}} + \text{glucose}_{\text{cyt}} + 5 \text{ADP}^{4-} + \text{AMP}^{2-} + \text{PP}^{4-} + 7 \text{P}^{4-} + 2 \text{CO}_2 + 2 \text{GDP}^{3-} + \text{NAD}^{+} + 7 \text{H}^{+} \\
(5)
\end{align*}
\]

The cost is the hydrolysis of nine “high-energy” phosphodiesteric bonds (admitting that the PP is hydrolyzed by inorganic pyrophosphatase). However, if the two cytosolic NADH are oxidized via the glycerophosphate shuttle, they yield three ATP molecules (10).

**Discussion**

In the last three decades, the remarkable advances of molecular biology and molecular genetics have somewhat eclipsed areas of traditional biology, such as enzymology and metabolism. In recent years metabolism has reemerged as a central topic in biology (4, 30, 31). Nevertheless, recurring errors create problems, when students try to balance the reactions of Tables 1 and 2 to develop an overall connection between ureagenesis and gluconeogenesis, including two modules. In the first module, students can use the single reactions of Tables 1 and 2 to develop an overall equation for each pathway. The section, *Introduction: the Equations of Urea Synthesis and of Gluconeogenesis*, contains most of the information related to this issue. In the second module, students will formulate an overall balanced equation encompassing both pathways. They can discuss the possible pathophysiological perturbation, the catabolism of the amino acids leading to fumarate and oxaloacetate, as well as the three carbons of odd chain fatty acids to succinyl-CoA during catabolism.

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**DISCLOSURES**

No conflicts of interest, financial or otherwise, are declared by the author(s).

**AUTHOR CONTRIBUTIONS**

P.L.I. and R.P. drafted manuscript; P.L.I. and R.P. edited and revised manuscript; P.L.I. and R.P. approved final version of manuscript; R.P. prepared figures.

**REFERENCES**


