A NEW EQUATION FOR CALCULATING THE NUMBER OF ATP MOLECULES GENERATED FROM FATTY ACIDS

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ABSTRACT

ATP, Adenosine triphosphate, is a complex entity requires for all organisms regardless of their size. All organisms such as bacteria and humans utilize ATP as a main source of their energy and a steady supply of ATP is so critical such that in poisoning cases such as cyanide poisoning the cells die as a result of protein production’s shutdown due to cyanide binding to the copper atom in cytochrome oxidase of the oxidative phosphorylation electron transport. This paper documents a novel approach for calculating the number of ATP molecules generated from oxidation of long chain saturated and unsaturated fatty acids with either even or odd numbers of carbon atoms. Our new approach is summarized in an equation based on the oxidation states of the different carbons of the fatty acids. Using this approach, the time required to calculate the number of ATP molecules generated from the oxidation of any long fatty acid is less than 2-3 minutes whereas in the regular, current, used approach the time needed is exceeding few hours. Furthermore, using the regular approach dictates the illustration of all fatty acid biochemical oxidation processes while our new approach documented herein does not.

KEYWORDS: Saturated fatty acids, unsaturated fatty acids, β-oxidation, ATP, adenosine triphosphate, Energy currency.
INTRODUCTION

ATP (nucleoside triphosphate) is a complex molecule consists of a nucleotide bound to phosphate groups and is utilized as a coenzyme by bacteria and humans cells.[1] In 1929, Karl Lohmann discovered ATP and in 1948 Alexander Todd was the first to synthesize this ubiquitous molecule.[2-3]

Energy is generally released from ATP molecules to do chemical, osmotic or mechanical work in the cell by a hydrolysis process by which ATP is hydrolyzed to ADP (adenosine diphosphate) and inorganic phosphate. Then the resulting ADP is immediately recycled in the mitochondria where it is recharged and becomes again as ATP.

ATP is not significantly unstable; however in the absence of a catalyst its hydrolysis is very slow to insure its stored energy. On the other hand, this stored energy in ATP is readily released in the presence of the appropriate enzyme.

ATP functions in a cyclic manner as a carrier of chemical energy from the catabolic reactions of metabolism to various cellular processes that require energy. It is utilized in a variety of functions including (i) transport work (osmotic work) in which different organic and inorganic substances are moved across cell membranes, (ii) mechanical work in which the energy is spent on muscle contraction such as in the cases of the heart, blood vessels, skeletal muscles and etc. This kind of work also applies on the work done by chromosomes and flagella to carry out their many different functions and (iii) chemical work in which the energy stored in ATP molecules is utilized to synthesize several thousands of macromolecules that the cell needs for its survival.[4-11]

ATP biosynthesis relies on utilizing the substances found in food as sugars, carbohydrates, fats and etc. The production of ATP by a non-photosynthetic aerobic eukaryote occurs in the mitochondria of cell by three major pathways: (1) glycolysis, (2) citric acid cycle/oxidative phosphorylation and (3) beta-oxidation.[4-11]

Fatty acids generally found in the form of triglycerides (in condensed and anhydrous shape) in humans are considered a very important source of ATP supply. 1 gram of fatty acids provides an approximately 9 kcal compared to only 4 kcal by carbohydrates. Due to the physicochemical properties of fatty acids which consist of a large lipophilic moiety, these
compounds can be stored in a relatively anhydrous environment and in a reduced size, whereas, carbohydrates are more highly hydrated, therefore their volume capacity is much higher.

Fatty acids contained in a variety of foods and are usually stored as triglycerides, which cannot be absorbed by the gastrointestinal tract.[12-18] Therefore, triglycerides are degraded into free fatty acids and mono-glycerides by the enzyme pancreatic lipase, found as a 1:1 complex with colipase protein. This protein is crucial for the activity of the enzyme pancreatic lipase. For an optimal activity of the complex, fatty acids should be in a water-fat environment. This is achieved by emulsifying the fatty acids by bile salts.

The products of triglycerides, free fatty acids and mono-glycerides, are absorbed through the intestinal wall along with a small fraction of free glycerol and di-glycerides. Once passed the intestinal wall, these substances are resynthesized into triglycerides and packaged into chylomicrons or lipoproteins, which are freed into the lacteals, and from there into the blood circulation. In the blood, these substances bind to the hepatocytes and adipocytes membranes or muscle fibers, where they are either oxidized for energy supply or stored.

Fatty acids can be further degraded to acetyl-CoA by the beta-oxidation process. In each round of beta-oxidation the length of the acyl chain is reduced by two carbon atoms and there is a production of one NADH and one FADH₂ molecules, which are utilized to generate ATP by the oxidative phosphorylation process. Since FADH₂ and NADH are characterized as energy-rich entities, tens of ATP molecules can be produced by the beta-oxidation of a single long fatty acid chain.[12-18]

The number of ATP molecules produced in cells from a fatty acid or a sugar is determined on the structural features of the substance; 1 mole of the fatty acid caprylic acid generates 61 moles of ATP, and 1 mole of oleic acid generates 144 moles, whereas 1 mole of glucose produces 36-38 moles of ATP. The current tool utilized for calculating the number of ATP generated from any fatty acid is time-consuming. It is estimated that several hours are needed for such calculations since many pathways and reaction steps are involved.[12-13]

In this manuscript, we report a novel approach for calculating the number of ATP molecules generated from any saturated or unsaturated fatty acid with either even or odd numbers of carbon atoms.
In the following text we demonstrate all pathways and steps taken into consideration when using the regular current method for calculating the number of ATP molecules generated from fatty acids.

Since free fatty acids have negative charge their ability to cross membranes is limited. Therefore, their permeability is largely dependent on specific transport protein, such as the SLC27 family fatty acid transport protein, which facilitates their membrane penetration. Upon reaching the cytosol, several processes should take place in order for the fatty acids to be transferred from the cytosol to the mitochondrial matrix where the beta-oxidation process takes place.[12-18]

In the mitochondrial matrix, fatty acids undergo beta-oxidation process in which two carbons are reduced from the fatty acid chain in every cycle to form acetyl-CoA. The beta-oxidation process involves four steps: (i) dehydrogenation of the fatty acid to trans-delta 2-enoyl CoA catalyzed by acyl CoA dehydrogenase, where the carbon-carbon double bond is formed between C2 and C3 and FAD molecule is reduced to FADH2 (Figure 1), (ii) hydration of trans-delta2-enoyl CoA to L-B-hydroxyacyl CoA catalyzed by enoyl-CoA hydratase (Figure 2), (iii) dehydrogenation of L-B-hydroxyacyl CoA to B-ketoacyl CoA catalyzed by B-hydroxyacyl CoA dehydrogenase enzyme in which the enzyme utilizes NAD as an electron acceptor (Figure 3), and (iv) thiolysis of B-ketoacyl CoA, between C2 and C3, catalyzed by thiolase enzyme. In this reaction a molecule of coenzyme A attacks C3 and breaks the bond to yield the first two carbons as acetyl CoA and a fatty acyl CoA with less two carbons (Figure 4). This process proceeds until all the fatty acid carbons are converted to acetyl CoA.[12-18]

There are different mechanisms for saturated fatty acids with an even number of carbons, saturated fatty acids with an odd number of carbons and unsaturated fatty acids. For even-numbered saturated fatty acids: each cycle of β-oxidation, generates a two carbon unit (acetyl-CoA), in a process of four steps. This process continues until the whole chain is degraded to acetyl CoA groups; the final cycle of the beta-oxidation yields two acetyl CoA groups instead of one acyl CoA and one acetyl CoA. For each cycle, the Acyl CoA group is shortened by two carbons. As a result of this process, one molecule of each of FADH2, NADH and acetyl CoA are formed. For odd-numbered saturated fatty acids: the fatty acid is oxidized in the same manner as even-numbered fatty acid, however, the final products of this
process are acetyl-CoA and propionyl-CoA. The Propionyl-CoA is then metabolized by several steps to form succinyl-CoA.

![Diagram](image1)

**Figure 1:** The first step in the beta-oxidation of fatty acids.

![Diagram](image2)

**Figure 2:** The second step in the beta-oxidation of fatty acids.

![Diagram](image3)

**Figure 3:** The third step in the beta-oxidation of fatty acids.

![Diagram](image4)

**Figure 4:** The fourth step in the beta-oxidation of fatty acids.

which then can enter the citric acid cycle. For unsaturated fatty acids: the β-oxidation poses a problem since the location of a cis bond can prevent the formation of a trans-Δ² bond. This problem is solved by the assistance of two enzymes, enoyl CoA isomerase or 2,4-dienoyl CoA reductase.[13-21]

**Calculations of ATP molecules generated from fatty acids[13-27]**

The total ATP yield for every beta-oxidation cycle is 17, as NADH generates 3 ATP, FADH₂ generates 2 and a full rotation of the citric acid cycle produces 12 (see Table 1).
Table 1: The number of ATP molecules generated in beta-oxidation process of fatty acids.

<table>
<thead>
<tr>
<th>SOURCE</th>
<th>ATP</th>
<th>TOTAL</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 FADH2</td>
<td>x 2 ATP</td>
<td>Theoretically 2 ATP</td>
</tr>
<tr>
<td>1 NADH</td>
<td>x 3 ATP</td>
<td>Theoretically 3 ATP</td>
</tr>
<tr>
<td>1 acetyl CoA</td>
<td>x 12 ATP</td>
<td>Theoretically 12 ATP</td>
</tr>
<tr>
<td>TOTAL</td>
<td></td>
<td>17 ATP</td>
</tr>
</tbody>
</table>

Therefore, for an even-numbered saturated fatty acids (C_{2n}); n - 1 oxidations are taking place and the final process yields an additional acetyl CoA. In addition, two equivalents of ATP are lost during the activation of the fatty acid. Therefore, the total ATP yield can be stated as:

(n - 1) * 17 + 12 - 2 = total ATP

For instance, the ATP yield of palmitic acid (C_{16}, n = 8) is:

(8 - 1) * 17 + 12 - 2 = 129 ATP (see Table 2).

For stearic acid (C_{18}), the ATP yield is:

(9 - 1) * 17 + 12 - 2 = 146 ATP

Table 2: Calculations of ATP molecules generated from the oxidation of palmitic acid (C_{16}).

<table>
<thead>
<tr>
<th>SOURCE</th>
<th>ATP</th>
<th>TOTAL</th>
</tr>
</thead>
<tbody>
<tr>
<td>7 FADH2</td>
<td>x 2 ATP</td>
<td>= 14 ATP</td>
</tr>
<tr>
<td>7 NADH</td>
<td>x 3 ATP</td>
<td>= 21 ATP</td>
</tr>
<tr>
<td>8 acetyl CoA</td>
<td>x 12 ATP</td>
<td>= 96 ATP</td>
</tr>
<tr>
<td>Activation</td>
<td></td>
<td>= -2 ATP</td>
</tr>
<tr>
<td>NET</td>
<td></td>
<td>= 129 ATP</td>
</tr>
</tbody>
</table>

In contrast to the lengthy and time-consuming approach described above for calculating the number of ATP molecules generated from a fatty acid, which dictates the necessity to illustrate all steps involved in the oxidation (degradation) process, the following novel approach summarized in equations 1-2 enables the calculations of the number of ATP molecules generated from any fatty acid within 1-2 minutes without the need to illustrate a fatty acid breakdown stages.

For even-numbered and unsaturated fatty acids.

\[(4+C_l) (nC_l) + (4+C_{LT}) (nC_{LT}) + (4+C_{RT}) (nC_{RT}) + (2.5xn) = N \quad (1)\]

For odd-numbered fatty acids:

\[(4+C_l) (nC_l) + (4+C_{LT}) (nC_{LT}) + (4+C_{RT}) (nC_{RT}) + (2.5xn) = N \quad (2)\]
Where $C_I$, $C_{LT}$ and $C_{RT}$ are the oxidation states of the internal, left terminal and right terminal carbons, respectively (Figure 5). $n$ is the number of carbons. $nC_I$, $nC_{LT}$ and $nC_{RT}$ are the number of internal, left terminal and right terminal carbons, respectively. $N$ is the number of ATP molecules generated.

The ATP calculations by equations 1-2 from any fatty acid are based on the oxidation states of the carbons of the fatty acid. For simplification, we illustrate the details for calculating the number of ATP molecules generated from palmitic acid as a representative of an even-numbered fatty acid (Figure 5).

![Chemical structure of palmitic acid (C16:0)](Image)

**Figure 5**: Chemical structure of palmitic acid (C16). The numbers in red are the oxidation states for the carbons of palmitic acid.

The oxidation states for $C_I$, $C_{LT}$ and $C_{RT}$ in palmitic acid are -2, +3 and -3. $n = 16$, $nC_I = 14$, $nC_{LT} = 1$ and $nC_{RT} = 1$ (Figure 5). Replacing $n$, $C_I$, $C_{LT}$, $C_{RT}$, $nC_I$, $nC_{LT}$ and $nC_{RT}$ with their corresponding values in equation 1 gives:

$$(4+2) \times 14 + (4+3) \times 1 + (4-3) \times 1 + 2.5 \times 16 - 3 = 129 \text{ ATP molecules}.$$  

The number of ATP molecules generated from unsaturated fatty acid can be calculated using equation 1. For example, the number of ATP molecules provided by the oxidation of myristoleic acid is:

$$(4+2) \times 10 + (4+3) \times 1 + (4-3) \times 1 + (4+1) \times 2 + 2.5 \times 14 - 3 = 110.$$  

On the other hand, the number of ATP molecules generated from an odd-numbered fatty acid can be calculated using equation 2. For example, the number of ATP molecules provided by the oxidation of margaric acid is:

$$(4+2) \times 15 + (4+3) \times 1 + (4-3) \times 1 + 2.5 \times 17 - 3.5 = 137 \text{ molecules}.$$  

Using equations 1-2, we have calculated the number of ATP molecules generated from different long chain saturated and unsaturated fatty acids with either even or odd numbers of carbon atoms and the results are illustrated in Table 3.
Table 3: Experimental and calculated ATP molecules generated from different fatty acids. *The calculated values were obtained using equations 1-2.

<table>
<thead>
<tr>
<th>Type of Fatty Acid</th>
<th>Fatty Acid Name</th>
<th>Chemical Structure</th>
<th>ATP Exp. Value</th>
<th>ATP Calc. Value</th>
<th>Calculations Details By Equations 1-2</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Odd saturated fatty acid</strong>[^23-24]</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Margaric acid</td>
<td>CH₃(CH₂)₁₅COOH</td>
<td>137</td>
<td>137</td>
<td>(4+2) x 15 + (4+3) x 1 + (4-3) x 1 + 2.5 x 17 -3.5 = 137</td>
<td></td>
</tr>
<tr>
<td>Pentadecylic acid</td>
<td>CH₃(CH₂)₁₃COOH</td>
<td>120</td>
<td>120</td>
<td>(4+2) x 13 + (4+3) x 1 + (4-3) x 1 + 2.5 x 15 -3.5 = 120</td>
<td></td>
</tr>
<tr>
<td><strong>Even saturated fatty acid</strong>[^13-22]</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Caprylic acid</td>
<td>CH₃(CH₂)₆COOH</td>
<td>61</td>
<td>61</td>
<td>(4+2) x 6 + (4+3) x 1 + (4-3) x 1 + 2.5 x 8 -3 = 61</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Capric acid</td>
<td>CH₃(CH₂)₈COOH</td>
<td>78</td>
<td>78</td>
<td>(4+2) x 8 + (4+3) x 1 + (4-3) x 1 + 2.5 x 10 -3 = 78</td>
<td></td>
</tr>
<tr>
<td>Lauric acid</td>
<td>CH₃(CH₂)₁₀COOH</td>
<td>95</td>
<td>95</td>
<td>(4+2) x 10 + (4+3) x 1 + (4-3) x 1 + 2.5 x 12 -3 = 95</td>
<td></td>
</tr>
<tr>
<td>Myristic acid</td>
<td>CH₃(CH₂)₁₂COOH</td>
<td>112</td>
<td>112</td>
<td>(4+2) x 12 + (4+3) x 1 + (4-3) x 1 + 2.5 x 14 -3 = 112</td>
<td></td>
</tr>
<tr>
<td>Palmitic acid</td>
<td>CH₃(CH₂)₁₄COOH</td>
<td>129</td>
<td>129</td>
<td>(4+2) x 14 + (4+3) x 1 + (4-3) x 1 + 2.5 x 16 -3 = 129</td>
<td></td>
</tr>
<tr>
<td>Stearic acid</td>
<td>CH₃(CH₂)₁₆COOH</td>
<td>146</td>
<td>146</td>
<td>(4+2) x 16 + (4+3) x 1 + (4-3) x 1 + 2.5 x 18 -3 = 146</td>
<td></td>
</tr>
<tr>
<td>Arachidic acid</td>
<td>CH₃(CH₂)₁₸COOH</td>
<td>163</td>
<td>163</td>
<td>(4+2) x 18 + (4+3) x 1 + (4-3) x 1 + 2.5 x 20 -3 = 163</td>
<td></td>
</tr>
<tr>
<td>Behenic acid</td>
<td>CH₃(CH₂)₂₀COOH</td>
<td>180</td>
<td>180</td>
<td>(4+2) x 20 + (4+3) x 1 + (4-3) x 1 + 2.5 x 22 -3 = 180</td>
<td></td>
</tr>
<tr>
<td>Lignoceric acid</td>
<td>CH₃(CH₂)₂₂COOH</td>
<td>197</td>
<td>197</td>
<td>(4+2) x 22 + (4+3) x 1 + (4-3) x 1 + 2.5 x 24 -3 = 197</td>
<td></td>
</tr>
<tr>
<td>Cerotic acid</td>
<td>CH₃(CH₂)₂₄COOH</td>
<td>214</td>
<td>214</td>
<td>(4+2) x 24 + (4+3) x 1 + (4-3) x 1 + 2.5 x 26 -3 = 214</td>
<td></td>
</tr>
<tr>
<td><strong>Unsaturated fatty acid</strong>[^26-27]</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Myristoleic acid</td>
<td>CH₃(CH₂)₃CH=CH(CH₂)₇COOH</td>
<td>110</td>
<td>110</td>
<td>(4+2) x 10 + (4+3) x 1 + (4-3) x 1 + 2.5 x 14 -3 = 110</td>
<td></td>
</tr>
<tr>
<td>Palmitoleic acid</td>
<td>CH₃(CH₂)₅CH=CH(CH₂)₇COOH</td>
<td>127</td>
<td>127</td>
<td>(4+2) x 12 + (4+3) x 1 + (4-3) x 1 + 2.5 x 16 -3 = 127</td>
<td></td>
</tr>
<tr>
<td>Sapienic acid</td>
<td>CH₃(CH₂)₈CH=CH(CH₂)₄COOH</td>
<td>127</td>
<td>127</td>
<td>(4+2) x 12 + (4+3) x 1 + (4-3) x 1 + 2.5 x 16 -3 = 127</td>
<td></td>
</tr>
<tr>
<td>Oleic acid</td>
<td>CH₃(CH₂)₇CH=CH(CH₂)₇COOH</td>
<td>144</td>
<td>144</td>
<td>(4+2) x 14 + (4+3) x 1 + (4-3) x 1 + 2.5 x 18 -3 = 144</td>
<td></td>
</tr>
<tr>
<td>Elaidic acid</td>
<td>CH₃(CH₂)₇CH=CH(CH₂)₇COOH</td>
<td>144</td>
<td>144</td>
<td>(4+2) x 14 + (4+3) x 1 + (4-3) x 1 + 2.5 x 18 -3 = 144</td>
<td></td>
</tr>
</tbody>
</table>
The results in Table 3 demonstrate overlapping between the calculated and experimental values of ATP generated from fatty acids. Hence, the use of equations 1-2 for calculating the oxidation of any fatty acid is fruitful, easy and fast.

**SUMMARY AND CONCLUSION**

Fatty acids are a sub-class of the lipid macronutrient class. One of the fatty acids major roles is energy production by supplying ATP (adenosine triphosphate). Fatty acids class is considered the highest energy carrier compared to other macronutrient classes such as proteins and carbohydrates. Fatty acids generate the most ATP on an energy per gram basis by the β-oxidation pathway. Energy is generally released from the ATP molecule to do chemical, osmotic or mechanical work in the cell by a hydrolysis process in which ATP is hydrolyzed to ADP (adenosine diphosphate) and inorganic phosphate. Then the resulting ADP is immediately recycled in the mitochondria where it is recharged and becomes again as ATP.

The regular approach used today for calculating the number of ATP molecules generated from an intake of a fatty acid (β-oxidation) is complex and time-consuming process that dictates an illustration of all pathways and steps involved in the fatty acid catabolism (β-oxidation). On the other hand, the novel approach reported herein and summarized in equations 1-2 is very simple and requires a very short time (less than 3 minutes) which can provide an easy tool to be utilized by students and researchers alike.

**REFERENCES**


