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# ANALYSIS OF METABOLIC NETWORKS OF SKELETAL MUSCLE CELL ENERGY METABOLISM

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Abstract: Network analysis was performed on the metabolic network of skeletal muscle cells where metabolites are denoted as nodes and reactions are denoted as connections. We also implemented an alternative approach that augments the metabolic network into an "interaction network" that represents influences that a certain metabolite can exert on others. With this novel method, key elements and modules of the system were identified with an emphasis on their regulatory function rather than just their contribution to material (carbon) flow within the pathways. We have utilized several different network analysis software packages to identify key metabolites, and motifs and investigated the modularity of these networks. 

Keywords: Biomedical systems, Networks, Network topologies

## 1. INTRODUCTION

Metabolic pathways are complex networks where each metabolite is connected to a handful of others via biochemical reactions. The metabolism exerts control over the network through regulation of the expression and activation of certain enzymes, leading to changes in mass fluxes. Mathematical models of cellular pathways should take into account such regulatory systems to capture the essential dynamics of the system. Often limited information and data are available about these mechanisms for modeling purposes. Key metabolites and regulatory components of the system can be identified via a comprehensive network analysis.

Diabetes is estimated to affect 6% of the adult population, afflicting over 200 million people worldwide by the end of the decade and Type 2 diabetes accounts for about 90 to 95 percent of 20.8 million diagnosed cases in the U.S. (CDC 2005). A better understanding of the alterations in insulin utilization in tissues by high levels of free fatty acids (FFA) will help identify the key steps to be targeted for treatment of Type 2 diabetes. Elevated plasma FFA and intracellular lipid concentration are the primary suspects for inhibiting glucose transport and causing insulin resistance in liver and muscle tissue (Sindelar *et al.* 1997, Dresner *et al.* 1999). Recent research has revealed important details about glucose and insulin metabolism and the effects of plasma FFA con-

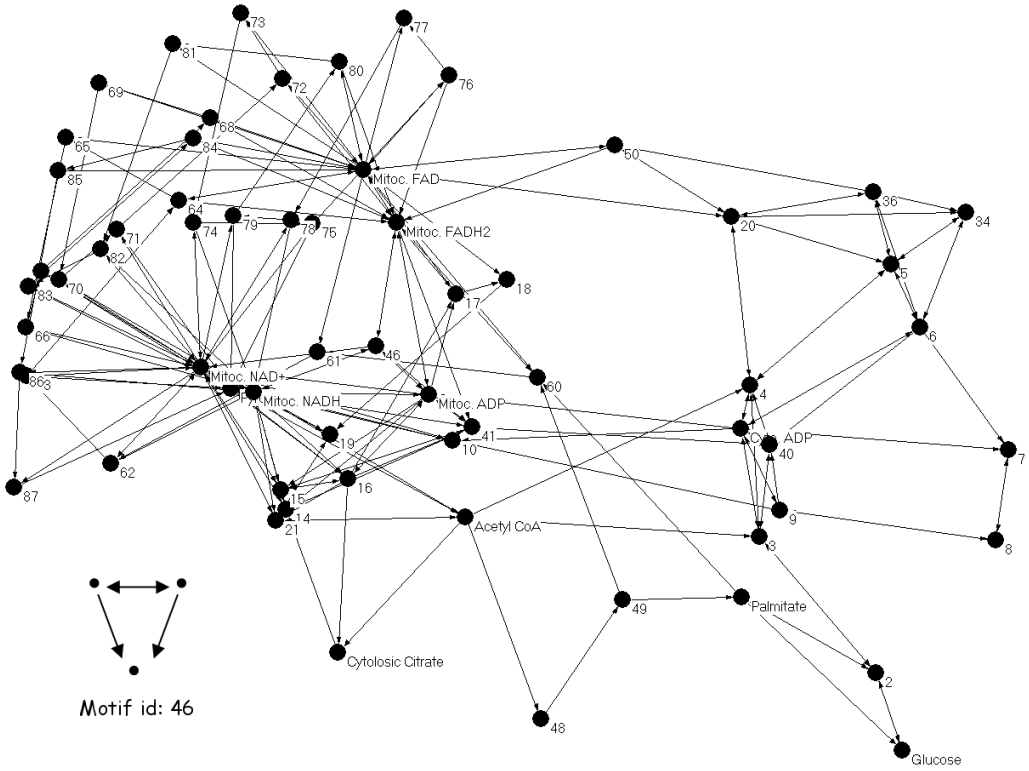


Fig. 1. Interaction network of skeletal muscle energy metabolism and its most dominant motif

centrations, however, detailed metabolic networks and dynamic models describing these phenomena are open research problems. To our knowledge, this research has been the first integrated approach utilizing a variety of system analysis and complex network analysis techniques to formulate and analyze metabolic networks and application of these methods to study the energy metabolism of muscle cells. The metabolic network of the pathways involved in the energy metabolism of muscle cells has been constructed to illustrate and test the methods and tools developed. System analysis has been performed to determine the overall connectivity within the system, and to identify key metabolites, regulatory structures and functional modules of the system.

We expect that these network development, modeling and system analysis tools will generate interesting research hypotheses for experimental and clinical studies on obesity and Type 2 diabetes, and reduce the experimental effort by conducting in-silico studies.

## 2. DEVELOPMENT OF METABOLIC NETWORKS FOR MODELING THE ENERGY METABOLISM OF MUSCLE CELLS

Metabolic networks can be visualized as nodes (substrates) connected to one another through links (metabolic reactions). The physical link is the temporary educt-educt complex in which enzymes provide the catalytic scaffolds for the reactions that yield products which in turn can become educts for subsequent reactions.

A reaction network of major pathways involved in energy metabolism of muscle cells has been constructed (Salway 2004). The network topology has been analyzed to investigate its modularity and to identify critical nodes (metabolites). Dominant motifs are determined by comparing the frequency of various functional motifs defined to those in randomized networks.

An alternative representation of the metabolic system was used, where the metabolic network was reconstructed as an "interaction network". Energy metabolism of skeletal muscle cell is a complex network where each metabolite can directly or indirectly influence the level of a handful of others via biochemical transformations and activation/deactivation of reactions. By utilizing current knowledge on such reactions and processes involved in the energy metabolism of skeletal muscle cells, we have developed the "interaction network" that maps the direct or indirect influences between metabolites (Roden *et al.* 1996, Opara 2005, Salway 2004)(Fig. 1). The influence of metabolite A on metabolite B is represented as an arrow from metabolite (node) A, pointing towards metabolite (node) B. The metabolites with key roles within the system were identified according

to their connection properties. Network motifs of the interaction network have been identified. Consistency of the results based on module and connectivity determination and motif identification has been assessed.

## 3. ANALYSIS OF METABOLIC NETWORKS OF SKELETAL MUSCLE CELLS

Analysis included identification of key metabolites according to their connectivity properties within the network, identification of motifs of reaction and interaction network and investigation of modularity of metabolic networks. Software used include mfinder (Kashtan *et al.* 2002) to find network motifs and UCInet (Borgatti *et al.* 2002) to analyze basic properties of the network and to identify its modules.

### *3.1 Identification of network motifs*

Certain sub-systems (composed of metabolites, biochemical reactions and interactions) within a metabolic network may serve specific functions in the system. Such sub-systems should be incorporated properly into the mathematical model. In case of insufficient experimental data, cybernetic modeling principles can be used to introduce the function of a certain segment into the mathematical model. We have identified motifs of the metabolic network, the patterns of interconnections occurring at numbers that are significantly higher than those in randomized networks. The significance of these structures raises the question of whether they have specific roles in the network. If they do, they might be used to understand the network dynamics in terms of elementary computational building blocks. Different networks belonging to a certain class (e.g. biological systems) may share the same unique network motifs. Therefore motifs can define broad classes of networks, each with specific types of elementary structures. Bi-fan and feed-forward loop motifs have been observed as common motifs of various biological systems (Milo *et al.* 2002).

Algorithm for motif identification in a network:

- The network is scanned for all 13 unique 3-node and 199 unique 4-node subgraphs.
- A randomized network for the same size and connection properties as the real network under investigation is developed.
- The number of occurrences of each subgraph in the real network is compared to the number of occurrences of the same subgraph in randomized networks of the same size and connection properties.
- Statistically significant subgraphs are determined as network motifs.

Motifs of the reaction network give insight to the common structures made by the paths of material (carbon) flow. 4-node, linear, irreversible structure is found to be the only significant motif, which points at the abundance of such structures within individual pathways (e.g. glycolysis, beta-oxidation). Glycogen metabolism has not been included in this analysis since it obviously yields the reversible linear motifs in large numbers and makes motifs of glycolysis, beta-oxidation and mitochondrial metabolism insignificant.

Since the interaction network maps the influences that a certain metabolite can exert on another, it represents regulation rather than just biochemical transformations within metabolic pathways. Therefore, the motifs identified in interaction network may potentially correspond to regulatory components in the system. The motifs we have identified for the interaction network of skeletal muscle cells are listed in Table 1.

Table 1. Number of occurrences ($N_{real}$) and significance (Z-score) of dominant motifs

| Motif ID | $N_{real}$ | Z-score |
|---|---|---|
| 46 | 25 | 8.22 |
| 110 | 40 | 5.34 |
| 238 | 17 | 2.29 |
| 94 | 139 | 3.58 |
| 414 | 22 | 4.33 |
| 328 | 155 | 3.39 |

### *3.2 Identification of key metabolites according to their connection properties within the reaction network and interaction network*

The metabolites with key roles within the system were identified according to their connection properties. We have analyzed the degree of emission (number nodes that a certain node can access) and the degree of reception (number of nodes that can access a certain node) after $n$ consecutive connections along the shortest path from the starting metabolite to the target metabolite.

A connection within the reaction network refers to a biochemical transformation, therefore, degree of emission represents the significance of a metabolite in terms of its ability to provide carbon for the production of different metabolites and degree of reception refers to the number of metabolites that play a role in producing a certain metabolite by providing carbon.

When $n$=1, the significance of a metabolite is characterized according the number of metabolites it directly provides with carbon and number of metabolites it directly receives carbon from via

a single biochemical reaction. In this case, the degree of emission and reception does not exceed 3 for any metabolite except for Acetyl-CoA which has a reception degree of 11 since it is directly produced from numerous steps within beta-oxidation pathway. By setting $n$=3 we define domains of metabolites to which a certain metabolite provides carbon and from which a certain metabolite receives carbon (Table 2, 3).

Table 2. Degree of emission and respective ranks within reaction network for $n$=3 (m: mitochondrial, rank: low number refers to high emission relative to other metabolites)

| Metabolite | Degree: Emission | Rank: Emission |
|---|---|---|
| Acetyl-CoA$_m$ | 5 | 20-31 |
| Citrate | 5 | 20-31 |
| Glucose 6-phosphate | 7 | 5-12 |
| Fructose 6-phosphate | 8 | 1-4 |
| 3-ketoacyl-CoA | 7 | 5-12 |

Table 3. Degree of reception and respective ranks within reaction network for $n$=3

| Metabolite | Degree: Reception | Rank: Reception |
|---|---|---|
| Acetyl-CoA$_m$ | 24 | 1 |
| Citrate | 20 | 2 |
| Glucose 6-phosphate | 8 | 6-7 |
| Fructose 6-phosphate | 9 | 5 |
| 3-ketoacyl-CoA | 3 | 25-69 |

For $n$=3 acetyl-CoA again has a very high degree of reception and since citrate is directly formed by the reaction of Acetyl-CoA and oxaloacetate it also has a high degree of reception through Acetyl-CoA. As indicated from high degree of emission and reception, glucose 6-phosphate contributes to the carbon flow to different metabolites significantly, which is indicative of its role as the central metabolite between glycolysis and glycogenesis pathways. Fructose 6-phosphate also has high degree of emission and reception due to its proximity to glucose 6-phosphate in the metabolic network. 3-ketoacyl-CoA, a metabolite in the beta-oxidation pathway, has a high degree of emission since it is a direct provider of carbons for the production of acetyl-CoA.

Since a connection within the interaction network corresponds to direct or indirect influence of a certain metabolite on another, degree of emission represents number of metabolites whose levels can be influenced by a certain metabolite and degree of reception represents number of metabolites that influence the level of a certain metabolite after $n$ influences. When $n$=1, immediate influences are highlighted. When $n$=3, a wider domain of influence is displayed.

The initial analysis was done by setting $n$=1. Cofactors such as NADH, $NAD^+$, FAD and $FADH_2$, were found to have high degree of emission and reception as expected since they appear as substrates or products in numerous reactions (Table 4, 5).

Table 4. Degree of emission and respective ranks within interaction network for $n$=1 (m: mitochondrial, c: cytosolic)

| Metabolite | Degree: Emission | Rank: Emission |
|---|---|---|
| Acetyl-CoA$_m$ | 2 | 42-51 |
| $NAD^+_m$ | 22 | 1 |
| NADH$_m$ | 12 | 3 |
| FAD$_m$ | 19 | 2 |
| FADH$_m$ | 4 | 14-17 |
| ADP$_m$ | 9 | 4 |
| ADP$_c$ | 8 | 5 |
| $O_2$ | 5 | 9-13 |
| X | 7 | 6 |

Table 5. Degree of reception and respective ranks within interaction network for $n$=1

| Metabolite | Degree: Reception | Rank: Reception |
|---|---|---|
| Acetyl-CoA$_m$ | 12 | 3-4 |
| $NAD^+_m$ | 15 | 1 |
| NADH$_m$ | 14 | 2 |
| FAD$_m$ | 10 | 5 |
| FADH$_m$ | 12 | 3-4 |
| ADP$_m$ | 6 | 6-9 |
| ADP$_c$ | 5 | 10-17 |
| $O_2$ | 3 | 23-29 |
| X | 4 | 18-22 |

Setting $n$=3 reveals the importance of dissolved oxygen and metabolite X in the system (Table 6, 7). The significance of oxygen and metabolite X can be observed by looking at their rapid climb in the ranks when $n$ is increased from 1 to only 3. Having a large domain of metabolites under which a certain metabolite is affected, may lead to the conclusion that the levels of this metabolite is tightly regulated by several other metabolites. Having a large domain of metabolites over which a certain metabolite exerts influence may lead to the conclusion that this metabolite is involved in the regulation of several other metabolites. In both cases, we can hypothesize that this metabolite serves a key role within the system.

Table 6. Degree of emission and respective ranks within interaction network for $n$=3

| Metabolite | Degree: Emission | Rank: Emission |
|---|---|---|
| Acetyl-CoA$_m$ | 36 | 33 |
| $NAD^+_m$ | 47 | 6 |
| NADH$_m$ | 46 | 7-8 |
| FAD$_m$ | 51 | 5 |
| FADH$_m$ | 54 | 1 |
| ADP$_m$ | 52 | 2-4 |
| ADP$_c$ | 46 | 7-8 |
| $O_2$ | 52 | 2-4 |
| X | 52 | 2-4 |

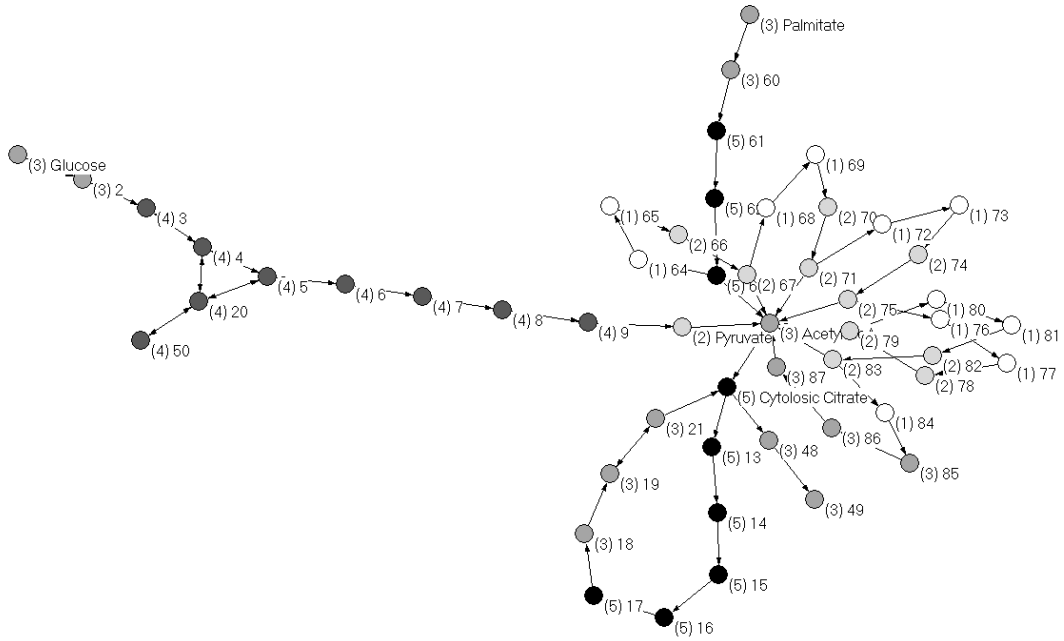


Fig. 2. Modules of interaction network represented on reaction network (Numbers in parenthesis refer to module numbers)

Table 7. Degree of reception and respective ranks within interaction network for $n$=3

| Metabolite | Degree: Reception | Rank: Reception |
|---|---|---|
| Acetyl-CoA$_m$ | 43 | 15-16 |
| NAD$^+_m$ | 53 | 4-5 |
| NADH$_m$ | 53 | 4-5 |
| FAD$_m$ | 39 | 25 |
| FADH$_m$ | 46 | 8 |
| ADP$_m$ | 59 | 1 |
| ADP$_c$ | 25 | 52-54 |
| O$_2$ | 54 | 2-3 |
| X | 45 | 9-12 |

### *3.3 Occurrences of highly connected metabolites in network motifs*

Highly connected metabolites (Table 4 and 5) of the interaction network are likely to be members of a large number of network motifs. The following analysis reveals the number of occurrences of each highly connected metabolite in each previously defined network motif.

Table 8. Occurrences of highly connected metabolites in network motifs

| Motif ID | N$_{real}$ | NAD$^+_m$ | NADH$_m$ | FAD$_m$ |
|---|---|---|---|---|
| 48 | 25 | 10 | 3 | 8 |
| 110 | 40 | 13 | 18 | 10 |
| 238 | 17 | 5 | 5 | 0 |
| 94 | 139 | 60 | 12 | 64 |
| 478 | 22 | 10 | 9 | 8 |
| 414 | 155 | 77 | 2 | 65 |
| 328 | 231 | 37 | 101 | 14 |
| Total | | 212 | 150 | 169 |

Table 8 shows that the certain highly connected metabolites appear mostly in certain network motifs (NAD$^+_m$ in motifs 94, 414 and 328, NADH$_m$ in motif 328 and FAD$_m$ in motifs 94 and 414). Since these metabolites usually perform a particular task in the system, the motifs that they are involved in are likely to be the structures representing these tasks, pointing out to the possibility that network motifs can be functional units.

### *3.4 Identification of clustering within the network and identification of modules*

We have analyzed the reaction network and interaction network to find a community structure in which network nodes are joined together in tightly knit groups between which there are only a few loose connections. A module of reaction network represents a group of metabolites that take part in a large number of biochemical reactions between each other compared to metabolites out of that group. Therefore modules of the reaction network can represent the metabolic pathways (e.g. glycolysis, glycogenesis) of the system.

Since the interaction network represents the influences that the metabolites exert on each other, identification of clusters may reveal functional modules, which may not refer to a certain pathway (e.g. glycolysis) but a collection of metabolites from different pathways working together to perform a specific task. A representation of

the reaction network numbered according to the functional modules found from interaction network displays the remoteness of the members of a functional module (Figure 2). Two important members of module number 3 are glucose and palmitate, which are shown to operate closely and influence each other readily within the system. Different members of the beta-oxidation, glycolysis pathways and even citric acid cycle can potentially play a role in regulating the utilization of glucose and palmitate based on their occurrence in the same functional modules.

### *3.5 Investigation of key metabolites according to their influence on different functional modules*

Metabolite X and oxygen have been identified as key metabolites according to the number of metabolites that may influence them and the number of metabolites which are likely to be influenced by them. Availability of oxygen is already known to be an important determinant of the system. To determine whether metabolite X may have an important role within the system, we have analyzed the domain of metabolites that may be influenced by metabolite X and oxygen and domain of metabolites which are likely to influence them to observe whether these domains contain metabolites from different functional modules. Both oxygen and metabolite X interact with a similar set of metabolites, which contains members from all functional modules and therefore metabolite X is potentially a key metabolite of the system.

## 4. CONCLUSION

Apart from the traditional method of representing metabolites and reactions as nodes and connections within a network, an interaction network was developed, which characterizes the interactions between metabolites rather than just biochemical transformations between them. Several techniques were applied for the analysis of reaction network and interaction network to identify key metabolites, network motifs, and functional modules. Results from different techniques were compared with each other to understand the role of key metabolites in regulatory motifs, and to differentiate the significance of key metabolites according to their roles in individual functional modules.

Energy metabolism of skeletal muscle was investigated with an emphasis on the interplay between the utilization of glucose and FFA. The techniques developed for construction and investigation of metabolic networks were used to identify the key metabolites, modules and regulatory components of the system. Identification of such components and metabolites can yield a better understanding of the alterations in insulin utilization in tissues caused by high levels of FFA and will help identify the key steps to be targeted for treatment of Type 2 diabetes.

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