Metformin Alters The Insulin Signaling Pathway In Ischemic Cardiac Tissue In A Swine Model Of Metabolic Syndrome

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Disclosures

- None
Background

- Metformin is one of the mainstay treatments of type 2 DM
- In addition to metformin’s glucose lowering effects, epidemiologic studies: metformin reduced all cause mortality\(^1,2\)
- Animal Studies of myocardial infarction and metformin\(^3\)
  - Reduce infarct size
  - Reduce hypertrophy
  - Reduce heart failure
  - Preserves myocardial function

Metformin’s cardioprotective effects are mediated by AKT and AMPK up-regulation, which activate the reperfusion injury salvage kinase pathway (RISK).

AKT and AMPK are also key protein kinases in the insulin signaling cascade.
Objective

- Investigate metformin at the junction of its cardioprotective and glucose lowering effects: the insulin signaling pathway.

- Clinically relevant swine model of metabolic syndrome to investigate the effects of insulin signaling in chronically ischemic myocardium.
The survival and insulin signaling pathways are intimately associated through AKT and AMPK.
Study Design

Ossabaw Mini-swine n=24

Regular diet n=8
High calorie n=16

9 weeks

Operation #1
Ameroid Placement

Operation #2
Blood Draw
Myocardial Perfusion
Cardiac Harvest

7 weeks

• Ossabaw Control OC n=8
• Ossabaw Hypercaloric OHC n=8
• Ossabaw Hypercaloric + Metformin OHCM n=8
Ameroid Constrictor Placement

1 – Dissection of left circumflex artery
2 – Placement of ameroid constrictor
BMI and Serum Insulin

- All animals were obese.
- OHC and OHCM groups had significantly higher BMIs compared to OC.
- OHC and OHCM groups had significantly higher serum insulin levels compared to OC.

* p<0.05
** p<0.01
Myocardial Perfusion

- Injected isotope-labeled microspheres into LA while occluding LCx at ameroid placement
- No differences in myocardial perfusion among groups.
<table>
<thead>
<tr>
<th>Target</th>
<th>OHC</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>IRS1</td>
<td>1.39±0.10</td>
<td>&lt;0.05*</td>
</tr>
<tr>
<td>pIRS1 (Ser 612)</td>
<td>1.43±0.10</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>IRS2</td>
<td>1.11±0.07</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>pIRS2 (Ser 731)</td>
<td>1.08±0.50</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>AMPKα</td>
<td>0.93±0.11</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>pAMPKα (Thr 172)</td>
<td>0.98±0.078</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>AKT</td>
<td>1.54±0.38</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>pAKT (Ser 473)</td>
<td>1.04±0.15</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>FOX01</td>
<td>1.48±0.15</td>
<td>&lt;0.05*</td>
</tr>
<tr>
<td>pFOX01 (Ser 256)</td>
<td>2.45±0.61</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>MTOR</td>
<td>1.40±0.27</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>pMTOR (Ser 2481)</td>
<td>1.59±0.36</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>PI3K</td>
<td>1.44±0.07</td>
<td>&lt;0.01*</td>
</tr>
<tr>
<td>RBP4</td>
<td>1.67±0.17</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>GLUT4</td>
<td>0.85±0.12</td>
<td>&gt;0.05</td>
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</tbody>
</table>

Fold change ± standard error of the mean compared to OC

* p value OC vs. OHC
Myocardial Protein Expression OHCM

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<th>p Value</th>
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</thead>
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<tr>
<td>IRS1</td>
<td>1.29±0.15</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>pIRS1 (Ser 612)</td>
<td>1.74±0.31</td>
<td>&lt;0.01 §</td>
</tr>
<tr>
<td>IRS2</td>
<td>1.25±0.08</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>pIRS2 (Ser 731)</td>
<td>1.43±0.05</td>
<td>&lt;0.01 § &lt;0.05 †</td>
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<tr>
<td>AMPKα</td>
<td>0.95±0.11</td>
<td>&gt;0.05</td>
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<tr>
<td>pAMPKα (Thr 172)</td>
<td>30.68±2.09</td>
<td>&lt;0.001 § †</td>
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<td>AKT</td>
<td>2.26±0.35</td>
<td>&lt;0.05 §</td>
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<tr>
<td>pAKT (Ser 473)</td>
<td>14.02±1.46</td>
<td>&lt;0.001 § †</td>
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<tr>
<td>FOX01</td>
<td>1.59±0.13</td>
<td>&lt;0.01 §</td>
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<tr>
<td>pFOX01 (Ser 256)</td>
<td>3.19±0.60</td>
<td>&lt;0.05 §</td>
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<tr>
<td>MTOR</td>
<td>2.54±0.19</td>
<td>&lt;0.001 § &lt;0.01 †</td>
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<tr>
<td>pMTOR (Ser 2481)</td>
<td>6.96±1.03</td>
<td>&lt;0.001 § †</td>
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<tr>
<td>PI3K</td>
<td>1.34±0.17</td>
<td>&lt;0.05 §</td>
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<tr>
<td>RBP4</td>
<td>2.70±0.58</td>
<td>&lt;0.01 §</td>
</tr>
<tr>
<td>GLUT4</td>
<td>1.06±0.22</td>
<td>&gt;0.05</td>
</tr>
</tbody>
</table>

Fold change ± standard error of the mean compared to OC

§ p value OC vs. OHCM
† p value OHC vs. OHCM
Phospho:Total Protein Expression Ratio

**IRS1**
- Phospho:Total IRS1
- p=0.095

**IRS2**
- Phospho:Total IRS2
- p=0.068

**MTOR**
- Phospho:Total MTOR
- p=0.181

**FoxO1**
- Phospho:Total FoxO1
- p=0.211

**AKT**
- Phospho:Total AKT

**AMPKα**
- Phospho:Total AMPKα

* p<0.001
Conclusions

- Metformin’s known cardioprotective mechanisms are independent of its glucose lowering effect but is intimately associated with the insulin signaling pathway.
- Chronic metformin significantly up-regulates the insulin signaling pathway in chronically ischemic myocardium in animals with metabolic syndrome.
Conclusions

- No change in GLUT4 expression despite dramatic metformin-mediated up-regulation of insulin signaling
- Metformin mediates up-regulation and plasma membrane translocation of GLUT1
- Previous Studies:
  - Insulin signaling and AMPK increase GLUT1 activity
  - GLUT1 over-expression protect cells from hypoxia induced apoptosis
- GLUT1 is another possible mechanism for metformin-mediated cardioprotection
Thank you

- Dr. Frank W. Sellke
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