Insulin/IGF-I Signaling

NEMATODE
Ligand
DAF-2
AGE 1
DAF-16

FLY
Ligand
Chico
Dp110/p60

MOUSE
Insulin/IGF-1
INR
INR β
IRS1-2
p85/p110 α–β
Forkhead transcription factor

HUMAN
Insulin/IGF-1
IGF1R/INSR
IRS1
p85/p110 α–β
HNF3/Forkhead

GLUCOSE METABOLISM
DEVELOPMENT
LONGEVITY
Insulin-like Properties of IGF-I

1. Binds to insulin and hybrid insulin/IGF-I receptors.
2. Stimulates glucose transport in skeletal muscle.
3. Inhibits hepatic glucose output.
4. Lowers blood glucose while simultaneously suppressing insulin.
Studies in Humans with Genetic Defects in Insulin or IGF-I Action
Retarded Growth of a Patient with a Deletion of the IGF-I Gene
# Changes in Insulin Sensitivity on IGF-I Therapy

<table>
<thead>
<tr>
<th>Age (yr)</th>
<th>IGF-I dose (ug/kg/day)</th>
<th>Duration of therapy (months)</th>
<th>Fasting insulin (mU/L)</th>
<th>Fasting glucose (mmol/L)</th>
<th>Si x10^-4 min^-1 (uU/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>16.07</td>
<td>0</td>
<td>0</td>
<td>28.9</td>
<td>4.8</td>
<td>1.45</td>
</tr>
<tr>
<td>16.15</td>
<td>40</td>
<td>1</td>
<td>14.2</td>
<td>5.1</td>
<td>2.06</td>
</tr>
<tr>
<td>16.55</td>
<td>80</td>
<td>6</td>
<td>7.5</td>
<td>4.7</td>
<td>4.39</td>
</tr>
</tbody>
</table>
Change During IGFI Treatment

Fructosamine (μM/mL)

HbA1c (%)

Time (0-16 months)
IGF-I in Type 1 Diabetes
Changes after Insulin

**IGF-I**

<table>
<thead>
<tr>
<th>Day</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
</tr>
</thead>
<tbody>
<tr>
<td>nmol/L</td>
<td></td>
<td></td>
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</table>

**IGFBP-1**

<table>
<thead>
<tr>
<th>Day</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
</tr>
</thead>
<tbody>
<tr>
<td>μg/L</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Free IGF-I levels in IDDM and Tanner stage matched controls
IGF-I in Type II Diabetes
PROTOCOL DESIGN: rhIGF-I and TYPE II DM

Experimental therapy

Usual Rx  No Rx  rhIGF-I (100 ug/kg bid sc)  Wash-out

Physiological testing  Physiological testing

Physiological testing
Modal Day
Mixed Liquid Meal
FSIVGTT
Change in Insulin Sensitivity on IGF1
SI Type II DM Subjects Before and After rhIGF-I Treatment

(80-100 μg/kg BID x 4-6 weeks)

- NIDDM
- rhIGF-I
- Women on BCPs
- Obese
- Aged, ad lib diet
- Mexican-Americans
- Aged, high CHO diet
- Postpartum
- Healthy women
- White men

Normal
Mechanisms By Which IGF-I Enhances Insulin Sensitivity

1. Direct lowering of glucose by IGF-I decreases glucotoxicity thus enhancing insulin action.

2. IGF-I / insulin hybrid receptor activation alters insulin-mediated signaling.

3. Lowering triglycerides and free fatty acids decreases their utilization for gluconeogenesis. This acts indirectly to facilitate glucose consumption in response to insulin.

4. Reduced counter-regulatory hormone production (e.g. glucagon and growth hormone) in response to IGF-I results in enhanced insulin action.
Suppression of Growth Hormone in type I DM
Insulin sensitivity index during each treatment period

* $P < 0.015$ compared to control
+ $P < 0.015$ compared to GHRA

JCEM 87:4356, 2002 O’Connell and Clemmons
## Study Design

### Study Design Table

<table>
<thead>
<tr>
<th>Screening</th>
<th>Pre-Rx ON therapy</th>
<th>Pre-Rx OFF therapy</th>
<th>Treatment Period</th>
<th>Post-treatment Period</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>80 μg/kg BID</td>
<td>F/U</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>40 μg/kg BID</td>
<td>F/U</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>20 μg/kg BID</td>
<td>F/U</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>10 μg/kg BID</td>
<td>F/U</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Placebo BID</td>
<td>F/U</td>
</tr>
</tbody>
</table>

8 Weeks | 2 Wks | 2 Wks | 12 Weeks | 12 Weeks
Effect of rhIGF-1 Treatment on Fasting Glucose

Change in Fasting Glucose (mg/dL)

Baseline: 294 mg/dL

Treatment Visits (Week)
Effect of rhIGF-1 Treatment on HbA1C

Change in Hemoglobin A1c (%)

Baseline HbA1c: 10.1%

Treatment Visits (Week)
F0685g Study Design

Treatment Period

- Insulin + Placebo/Placebo
- Insulin + rhIGF-I 20/20*
- Insulin + rhIGF-I 40/40*
- Insulin + rhIGF-I 80/40*

Post Treatment

- F/U
- F/U
- F/U
- F/U

4 Weeks | 2 Weeks | 12 Weeks | 2 Weeks

Instruction on blood glucose monitoring and intensified insulin regimen

*rhIGF-I in μg/kg BID

CRC F0685e 12/18/96
## Selected Adverse Events

<table>
<thead>
<tr>
<th>Preferred Term</th>
<th>Placebo (n=43)</th>
<th>10 ug/kg (n=41)</th>
<th>20 ug/kg (n=44)</th>
<th>40 ug/kg (n=41)</th>
<th>80 ug/kg (n=43)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jaw pain</td>
<td>4.7%</td>
<td>7.3%</td>
<td>9.1%</td>
<td>19.5%</td>
<td>51.2%</td>
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<tr>
<td>Face edema</td>
<td>2.3%</td>
<td>7.3%</td>
<td>2.3%</td>
<td>12.2%</td>
<td>25.6%</td>
</tr>
<tr>
<td>Generalized edema</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peripheral edema</td>
<td>7.0%</td>
<td>9.8%</td>
<td>4.5%</td>
<td>29.3%</td>
<td>25.6%</td>
</tr>
<tr>
<td>Hypoglycemia</td>
<td>11.6%</td>
<td>2.4%</td>
<td>4.5%</td>
<td>7.3%</td>
<td>4.7%</td>
</tr>
<tr>
<td>Dizziness</td>
<td>11.6%</td>
<td>12.2%</td>
<td>11.4%</td>
<td>9.8%</td>
<td>18.6%</td>
</tr>
<tr>
<td>Arthalgia</td>
<td>7.0%</td>
<td>7.3%</td>
<td>6.8%</td>
<td>14.6%</td>
<td>11.6%</td>
</tr>
<tr>
<td>Paresthesia</td>
<td>14.0%</td>
<td>12.2%</td>
<td>13.6%</td>
<td>17.1%</td>
<td>32.6%</td>
</tr>
<tr>
<td>Facial paralysis</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>2.3%</td>
</tr>
<tr>
<td>Myocardial infarct</td>
<td>2.3%</td>
<td></td>
<td></td>
<td>2.3%</td>
<td>2.3%</td>
</tr>
<tr>
<td>Tachycardia</td>
<td>2.3%</td>
<td></td>
<td></td>
<td></td>
<td>9.3%</td>
</tr>
<tr>
<td>Pseudotumor cerebrii</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>2.3%</td>
</tr>
<tr>
<td>Liver abnormalities</td>
<td>2.3%</td>
<td></td>
<td></td>
<td></td>
<td>2.3%</td>
</tr>
</tbody>
</table>
Free IGF-I levels obtained at serial intervals during 3 hours following the first rhIGF-I / placebo injection.
TOTAL SERUM IGF-I

Study day

IGF-I (ng/ml)

Placebo (n=4)
0.125 mg/kg (n=5)
0.25 mg/kg (n=5)
0.50 mg/kg (n=7)
0.50 mg/kg b.i.d. (n=5)
1.0 mg/kg (n=5)
2.0 mg/kg (n=6)
# Summary of Complications

<table>
<thead>
<tr>
<th>Complication</th>
<th>IGF-I Alone (100-160 ug/kg/day)</th>
<th>IGF-I/IGFBP-3 (440 ug/kg/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peripheral edema</td>
<td>39%</td>
<td>14%</td>
</tr>
<tr>
<td>Jaw pain</td>
<td>47%</td>
<td>3%</td>
</tr>
<tr>
<td>Arthralgias</td>
<td>33%</td>
<td>16%</td>
</tr>
<tr>
<td>Myalgias</td>
<td>`24%</td>
<td>11%</td>
</tr>
<tr>
<td>Palpitations</td>
<td>11%</td>
<td>2%</td>
</tr>
<tr>
<td>Optic nerve edema</td>
<td>8%</td>
<td>2%</td>
</tr>
<tr>
<td>Back Pain</td>
<td>27%</td>
<td>14%</td>
</tr>
<tr>
<td>Nausea</td>
<td>14%</td>
<td>13%</td>
</tr>
<tr>
<td>Injection site irritation</td>
<td>7%</td>
<td>18%</td>
</tr>
<tr>
<td>Hypoglycemia</td>
<td>22%</td>
<td>20%</td>
</tr>
</tbody>
</table>
Effect of Somatokine on Mean Blood Glucose

Group 1: 2mg/kg/day (cont. s.c. infusion)

Group 2: 2 mg/kg/day (6 hr o.n. s.c. infusion)

Group 3: 2 mg/kg/day (s.c. injection bid)

Group 4: 1mg/kg/day (s.c. injection qhs)

Day 0  Day 13/14 Average
IGF-I Stimulates Migration and Proliferation In the Presence of High but not Normal Glucose
Diabetic Retinopathy

Patients with proliferative retinopathy (70%) do not respond to VEGF inhibitors.

Acellular capillaries/EC dysfunction pericyte drop-out increased vascular permeability.
Protocol

Citrate control
Rats n = 12

STZ 50mg/kg
Rats n = 12

STZ 50mg/kg
Rats n = 12

Confirm the rats are diabetic and wait two weeks

IP injections /72 hrs PBS

IP injections /72 hrs PBS

IP injections /72 hrs C-loop Ab

2 weeks of antibody /control treatment

Examine vascular permeability by measuring leakage of dye from the retinal vasculature
Vascular leakage combined data

- Control (n=37)
- Hyperglycemic (n=31)
- Hyperglycemic + B6 (n=23)
- Hyperglycemic + C loop (n=17)

Evans blue permeation (ul plasma x g retina dry wt-1 x hr)

- Control (n=31)
- Diabetic (n = 31)
- Diabetic + C-loop (n = 17)
IB: Occludin

IB: β3

Blood-side

Capillary endothelium

Tight junctions

Occludin
Claudin
JAM
ECSAM
VE-cadherin

Actin

Catenin

Adherens junctions

VEGF
IGF-I
C-loop
Integrin and IGF-I signaling

αVβ3

Vn

IGF-I

IGF-IR

SHP-2

SHP-2

DOK-1

DOK-1

Nox4

Grb2

c-Src

SHC

SHPS-1

+ IGF-1

+ IGF-1

+ IGF-1

signaling
Coordination between the stimulatory signals arising from nutrients and growth factors
Knockdown of AMPK Enhances IGF-I Signaling.

Ning, J. et al. Mol Endocrinol 2010;24:1218-1229
IGF-I suppresses metformin-induced AMPK activation in an AKT dependent manner
Knockdown AKT enhances basal AMPK activation and impairs the ability of IGF-I to suppress AMPK activation
AMPK S485D prevents metformin-induced AMPK activation while AMPK S485A allows metformin-induced AMPK activation
AMPK S485D impairs AMPK activation and enhances IGF-I-stimulated P70S6K activation
Obese and Lean AMPK activation

Phospho-AMPK(T172)
Phospho-P70s6k(T389)
Total-AMPK
IGF-I

Obese
Lean

- + + + - + + +
0 5 10 20 0 5 10 20 min
IGF-I Stimulation of PI3K Pathway in Lean and Obese Pigs

**IB: mTOR(S2448)**

**IB: 4E-BP1(S65)**

**IB: mTOR**

0 10 0 10 min p IGF-I

Lean Obese

**PI-3 Kinase Activity**

**IP: p85**

0 10 0 10 min p IGF-I

Lean Obese
Muscle Protein Synthesis

3H - Leucine Incorporation (CPM x 10^-3)

IGF-I Dose (μg/kg/LBM)

Lean
Obese

Muscle Protein Synthesis

3H - Leucine Incorporation (CPM x 10^-3)

IGF-I Dose (μg/kg/LBM)

Lean
Obese
Bidirectional Signaling Between AMPK and IGF-I

IGF-I

IGF type-I receptor

IRS-1

AKT

IRS-1

S794

Anti apoptosis, protein synthesis

AMPK

T172

S485

AMPK

S485
Summary

• IGF-I enhances insulin sensitivity in patients with type 2 diabetes
• IGF-I significantly lowered HBA1C in patients who were receiving insulin
• IGF-I induced side effects are dose dependent and reversible
• The window between the free IGF-I concentration required for efficacy and the level that induces toxicity is narrow.
• Inhibition of AMPK and preferential stimulation of selected cell types may limit effectiveness