Gene Transcription; A Pathway for Increasing ApoA-I Protein Production.

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Speaker Disclosures;

1. Chief Scientific Officer, Resverlogix Corp. (RVX)
2. Holder of shares & options in RVX
Topics to be presented;

1. Background; apoA-I and HDL
2. Thyroid hormone regulation of apoA-I transcription.
3. Tissue specific apoA-I expression.
4. Insulin and Glucose control of apoA-I transcription.
5. Summary
Plasma HDL Concentration and Development of Ischaemic Heart-Disease and HDL Hypothesis

G. J. Miller
Medical Research Council Pneumoconiosis Unit,
Llandough Hospital, Penarth, South Wales

N. E. Miller
Department of Cardiology and Lipid Research Laboratory,
Royal Infirmary, Edinburgh, Scotland

In accordance with this hypothesis, it is further proposed that the development of atherosclerosis might be more successfully prevented by increasing plasma-H.D.L, and hence the clearance of cholesterol from the arterial wall, than by conventional attempts to reduce the plasma cholesterol and other lipoproteins.

Fig. 3—Lipoprotein cholesterol concentrations in eight patients with I.H.D. but normal plasma-total-cholesterol concentration (<250 mg. per 100 ml.) and fourteen healthy controls.††
Ages: I.H.D. 54±1 years; controls 51±2 years (mean ±S.E.M.).
ApoA-I is the major protein component of HDL
Steps Where HDL Metabolism may be Affected.

- Cholesterol
- LDL-R
- SR-B1
- ApoA-1
- RVX-208
- ApoA-IMimetics
- LXR
- PPAR
- RXR/RAR
- Niacin
- CETP
- inhibitors

Foam Cell

bile

ABCA1

RVX-208 ApoA-I Mimetics

RCT

ABCG1

LCAT

CE

α-HDL

CETP

VLDL

LDL-R

SR-B1

ABCA1

Foam Cell

Niacin

??
Rationale underlying our interest in apoA-I

Increasing apoA-I production should make more young HDL particles which in turn should raise the levels of mature HDL.

1.) apoA-I is the major protein component and the rate limiting precursor of HDL

2.) Pre-β or lipid poor HDL has highest potency in cholesterol efflux activity
Cholesterol Efflux Capacity, High Density Lipoprotein Function and Atherosclerosis

Table 3. Coronary Artery Disease Status According to Quartile of Efflux Capacity.

<table>
<thead>
<tr>
<th>Variable</th>
<th>No. of Patients</th>
<th>Odds Ratio for Coronary Artery Disease (95% CI)*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Adjusted for Cardiovascular Risk Factors</td>
<td>Adjusted for Cardiovascular Risk Factors and HDL Cholesterol</td>
</tr>
<tr>
<td>Quartile 1</td>
<td>198</td>
<td>1.00</td>
</tr>
<tr>
<td>Quartile 2</td>
<td>198</td>
<td>0.75 (0.48–1.16)</td>
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<tr>
<td>Quartile 3</td>
<td>198</td>
<td>0.58 (0.37–0.89)</td>
</tr>
<tr>
<td>Quartile 4</td>
<td>199</td>
<td>0.40 (0.25–0.63)</td>
</tr>
<tr>
<td>P value for trend</td>
<td>&lt;0.001</td>
<td>0.002</td>
</tr>
</tbody>
</table>

* Cardiovascular risk factors included in the logistic-regression model were age, sex, smoking status, presence or absence of diabetes, presence or absence of hypertension, and low-density lipoprotein cholesterol. HDL denotes high-density lipoprotein.
Acute ApoA-I infusion therapies decrease atheroma

![Graph showing the decrease in total atheroma volume (TAV) for different groups: Delipidated HDL (n=28), Control (n=47), ApoA-I Wt Milano (n=47), compared to the control group.](image)
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Regulatory elements within the apoA-I promoter

IRCE

Sp1
(In insulin + glucose -)

A

TR
HNF-4
ARP-1
RAR
RXR
PPAR
(Hormonal regulation)

B

HNF-3αβγ
Egr-1
(Age related)

C

HNF-4
ARP-1
(Tissue specific)
Thyroid hormone induces hepatic apoA-I (S11) mRNA activity

L-triiodothyronine (T3)

Hypothyroid
Hypothyroid + 15 µg/kg T3
Hypothyroid + 200 µg/kg T3

Euthyroid
Thyroid hormone induces rat ApoA-I mRNA in Liver and Small Intestine
Activity of Rat Apo Al Promoter in Liver Cells

![Diagram of Apo Al promoter activity with various transcription factors and binding sites.]

- IRCE
- Sp1
- T3R
- HNF-4
- ARP-1
- RAR
- RXR
- PPAR
- HNF-3 αβγ
- Egr-1
- HNF-4
- ARP-1

-144, -186, -232, -474 positions relative to the transcription start site.

[Images of gel electrophoresis results for HuH-7 and huH-1 cell lines with bands indicated for each sample.]
T3 Induction of Apo AI Promoter Activity Requires Site A

ACCTGA-AC-CCTTGA
Structures of L-triiodothyronine (T3) and a Thyromimetic CGS-23425

L-triiodothyronine (T3)

CGS-23425
Differential effect of CGS 23425 and L-T3 on apoA-I promoter activity in the presence of T3R-β/-α.
CGS 23425 raises apoA-I but also lowers both LDL and total cholesterol.
Both L-T3 and CGS-23425 are small molecules that increases apoA-I gene transcription.
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An Apo Al Activator and Repressor Regulate Expression at Site C

- Sites C and A shared similarities
- Binds to members of the nuclear receptor superfamily
- ARP-1 and HNF-4 tested

\[
\begin{align*}
\text{AGCTGA} & \quad T \quad \text{CCTTGA} \quad (C) \\
\text{ACCTGA} & \quad \text{AC} \quad \text{CCTTGA} \quad (A)
\end{align*}
\]
Orphan Receptors HNF-4 Stimulates but ARP-1 Represses Apo AI Promoter Activity at Site C
Both HNF-4 and ARP-1 Bind to Overlapping Motifs in Site C

![Diagram showing the binding of HNF-4 and ARP-1 to overlapping motifs in Site C.](image)

**A**
- Extracts: rHNF, Control, HNF-4, ARP-1
- lanes: 1, 2, 3, 4
- Sites: A, B, C, D

**B**
- HNF-4
- lanes: 1, 2, 3, 4, 5, 6, 7
- Competitor DNA: None, Wt, Cm-r, Cm-2, Cm-3, Cm-4, Cm-5

**C**
- ARP-1
- lanes: 1, 2, 3, 4, 5, 6, 7
- Competitor DNA: None, Wt, Cm-1, Cm-2, Cm-3, Cm-4, Cm-5
Site C Binds to ARP-1 and HNF-4

- Whereas HNF-4 activates, ARP-1 represses apo Al gene activity
- Both factors bind to overlapping parts of the same motif
- Both are orphan receptors of the same nuclear receptor superfamily
- HNF-4 is found in liver and small intestine, where apoA-I is expressed but ARP-1 is everywhere.
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Diabetes & Dyslipidemia

• Combined (mixed) dyslipidemia common

Insulin deficiency and resistance impair activity of lipoprotein lipase (LPL), which hydrolyzes TG

• TC & LDL-C: frequency similar to individuals without diabetes

**Insulin increases but glucose decreases apo AI protein and mRNA in Hep G2 cells**

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<tr>
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<th>24 Hours</th>
<th>48 Hours</th>
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<tr>
<td>Control</td>
<td>1, 2</td>
<td>5, 6</td>
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<tr>
<td>Insulin</td>
<td>3, 4</td>
<td>7, 8</td>
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<tr>
<td>Insulin + Glucose</td>
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**apoA-I protein-Western**

**apoA-I mRNA-Northern**

1, 5 Control Media
2, 6 Glucose
7, 3 Insulin
4, 8 Insulin + Glucose
Insulin increases but glucose decreases Apo AI Promoter Activity

Control Media  Glucose  Insulin  Insulin+ Glucose

-474  -7

CAT  pAl.474.CAT

1  2  3  4

Relative CAT-Activity

Control Media  Glucose  Insulin  Insulin+ Glucose

0  50  100  150  200  250

*
Deletion of -425 to -376 leads to lost of response to insulin and glucose

A

-474 CAT pAI.474.CAT
-425 CAT pAI.425.CAT
-375 CAT pAI.375.CAT

B

Relative CAT-Activity

Control Media Glucose Insulin Insulin+ Glucose

C

Relative CAT-Activity

pAI.425.CAT

Control Media Glucose Insulin Insulin+ Glucose

D

Relative CAT-Activity

pAI.375.CAT

Control Media Glucose Insulin Insulin+ Glucose
Mutation of the insulin responsive core element (IRCE) eliminates response to insulin and glucose.

-425
-7
CAT

**Control** Glucose Insulin Insulin + Glucose

- Mutant: TGCAACGAAACTTTttttttttGATGTGAGTTTCAGG
- Wild type: TGCAACGAAACTTTGAGGGGGATGTGAGTTTCAGG

**Relative CAT Activity**

- Sp1
- T3R
- HNF-4
- ARP-1
- RAR
- RXR
- PPAR
- HNF-3 αβγ
- Egr-1
- HNF-4 ARP-1

**pmAI.425.CAT**

**pAI.425.CAT**

* denotes significant difference.
Sp1 Binds to the IRCE

A

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<tr>
<th>FSK</th>
<th>PDBu</th>
<th>Nuclear protein</th>
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B

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C

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<tr>
<th>Antibody</th>
<th>Sp1 Ab</th>
<th>Nuclear protein</th>
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5. Summary
Summary;

1. Increasing apoA-I production should enhance HDL functionality.
2. Thyroid hormone, a small molecule increases apoA-I transcription.
3. HNF-4 augments but ARP-1 represses tissue specific transcription.
4. Insulin increases but Glucose inhibits apoA-I gene transcription.
5. Thus apoA-I gene transcription can be regulated by small molecules and this finding underlies the search of others to increase apoA-I production.

Acknowledgements;
1. Jeannie Chan, Ph.D
2. Jacques Romney, M.D.
3. Tony Taylor, M.D., Ph.D
4. Koji Mauro, M.D., Ph.D
5. Johnny Lam, Ph.D
**Cardiac Sparing Actions of CGS23425**

Table 2. The effect of CGS 23425 on the cardiac activity of euthyroid rats.

<table>
<thead>
<tr>
<th>Dose (mg/Kg)</th>
<th>Heart Weight (% of Body wt)</th>
<th>Atrial Contractility (beats/min)</th>
<th>Atrial Force (% of initial)</th>
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</thead>
<tbody>
<tr>
<td>0</td>
<td>0.37 ± 0.01</td>
<td>191 ± 6</td>
<td>87 ± 2</td>
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<tr>
<td>2.5</td>
<td>0.35 ± 0.02</td>
<td>198 ± 10</td>
<td>81 ± 4</td>
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<tr>
<td>10</td>
<td>0.40 ± 0.01</td>
<td>210 ± 14</td>
<td>84 ± 8</td>
</tr>
<tr>
<td>40</td>
<td>0.44 ± 0.02*</td>
<td>150 ± 5*</td>
<td>102 ± 4*</td>
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</table>
Human and Rat Apolipoprotein AI Promoter

rat -464 ...........................................GGAGACC -458
hum -495 AGAGGCTGAGGACCTGTGGGACTAAAGAAGAGCACTGGTGGGAGGAC -445
rat -457 AGGGTGACGAAAAGGGGAAACTCTCTTG........GAATGCAACGAAACTTT
hum -444 AGGGCGGGG.G.AAGGGGGAGGGAGTGAAGTAGT.CACTGGTGGGATCTG
rat -411 GAGGGCCGGG.ATGTGAGT.TCAGGAGCCAAATCTCGACTTCCTCTCTCTAC
hum -396 GGGTGGGAGGG.ATGTGAGT.TCAGGAGCCAAATCTCGACTTCCTCTCTAC
rat -363 CCTGCTCCAGTTCCAGGGCAGTACACAGACAGGGAGCCAGGGAGTTGAC
hum -348 CCTCCCC.....TC.....CTCTGCAACGAAACTGGGACATGGGA
rat -313 TACATGAGACTAACTAAAAGAACACATATACCGCAGGATGGGGCAACTGC
hum -307 CACACACTCCCATGGGAGGGATGATGGCAGCTGCTGGGACCTGC
rat -263 CTTACCCCCTGGG.GACCCTTGGAGTCTGCAGCTACTCCCCC.CTGCCCC
hum -263 GACCCCACCCGGGAGACCTGCA.AGCCTGCAGACACTCCCCTCCCGCCCC
rat -215 CACCTGAACCCTTGATCCCAGCTCTGCAGCCCCCGCAGCTTCCTGTTTG
hum -214 CACCTGAACCCTTGATCCCAGCTCTGCAGCCCCCGCAGCTTCCTGTTTG
rat -165 CCCACTCTGTTTGCCTAGCCTCGGGAACAGAGCTGATCCTTGAACTCTAA
hum -166 CCCACTCTATTTGCCCAGCCCCAGGGACAGAGCTGATCCTTGAACTCTTA
rat -115 GTGACCCGACCCAGCAAAATGAAGCAGGTACAGGCCAGCCAGCCTGAGCT
hum -116 GTGACCCGACCCAGCAAAATGAAGCAGGTACAGGCCAGCCAGCCTGAGCT
rat -66 ATCAGCTCTCCAGCCAGCCAGCCAGCCAGCCAGCCAGCCAGCCAGCCAGCCAG
hum -66 ATCAGCTCTCCAGCCAGCCAGCCAGCCAGCCAGCCAGCCAGCCAGCCAGCCAG
rat -16 GAAGACCTGGACACCCA +1
hum -16 GA.G..CTGGGTGCTTAGAG +1

Element
Position relative to Rat +1

IRCE -411 - 404  Insulin/glucose

SP1 -228 - 214
Egr-1 -221 - 212
Site A -208 - 193
Site S -186 - 171
Site B -170 - 145
Site C -130 - 116
CCATT Box -112 - 107
Site D -68 - 62
TATA -32 - 25
nTRE -27 - 22
T3 Induction of Apo Al Promoter Activity Requires Site A

ACCTGA-AC-CCTTGA