Genetic variability of adiponectin’s gene affects arterial redox state by regulating adiponectin biosynthesis in adipose tissue from patients with atherosclerosis


1st Cardiology Dpt, Hippokration Hospital, Athens - Greece
Adiponectin

Autocrine
Adiponectin
Prevents insulin resistance
Adipose tissue

Endocrine
Paracrine
VSMC
Proliferation
Migration

Endothelium

Vascular effects
†NO synthesis
(†eNOS activity†eNOS expression)
↓TNF-α induced ADMA production
Antioxidant effects
(↓NADPH-oxidase activity →↓O2-)
↓Expression of adhesion molecules

C Antoniades, et al Obes Rev 2009;10;269-279
Adiponectin in clinical studies

Adiponectin
Protective effect
in healthy subjects

High adiponectin
Poor prognosis in
CVD patients

Healthy subjects

Koenig et al, JACC 2006; 48:1369–77

CAD patients
(preserved LVF)
(n=1890)

HF patients
(n=449)
Adiponectin genetic variability

- ADIPOQ: chromosome 3q27

T45G: Paracrine effect?
G276T: Endocrine effect?

IR, OBESITY, T2DM, CAD risk, Blood pressure, LDL-particle size
Study hypothesis

Adiponectin

Vascular mechanics and NO-mediated vasorelaxations

Adiponectin synthesis

Adiponectin

NADPH-oxidase

Endothelial cell

NADPH-oxidase

VSMC

\( \downarrow \text{O}_2^- \) \( \uparrow \text{NO} \)

\( \downarrow \text{ONOO}^- \)

Endothelial cell

Adiponectin

ATHEROGENESIS??

NADPH-oxidase

VSMC

Paracrine
Aim of the Study

By using the presence of functional genetic polymorphisms on adiponectin gene (T45G & G276T) as a model of chronic hypo- or hyper-adiponectin expression in human adipose tissue:

a) we examined the effects of adiponectin on vascular redox state in human arteries and explored the underlying mechanisms

b) we evaluated the effects of adiponectin on NO bioavailability and vascular mechanics

in patients with coronary atherosclerosis
Study Design (part A)

169 patients undergoing elective CABG

Before CABG

Blood Sampling

Endothelial function

Arterial distensibility

During CABG

Internal mammary arteries (IMA)

Intrathoracic Adipose Tissue

Transferred within 30 min

in the Lab

Discharged

Genotyping

Serum adiponectin

Vascular $O_2^-$ generation

(Lucigenin chemiluminescence)

Adiponectin biosynthesis

(Ex-vivo AT culture at 37°C for 4h)
**Methods**

T45G and G276T genotypes were determined by PCR.

Endothelial function was determined by flow mediated dilatation of brachial artery (FMD) using high resolution U/S (% change in baseline diameter at 60sec post cuff release).

Arterial Distensibility was determined by the brachial artery diameter waveform analysis and systemic blood pressure (assessed by standard cuff–pressure sphygmomanometry).
Vascular $O_2^-$ generation in IMA rings was determined ex-vivo using lucigenin-enhanced chemiluminescence in the presence or absence of NADPH (100μmol/L), as we have described in the past.  

Antoniades C et al, Circulation 2009

AT samples were cultured ex-vivo at 37°C for 4 hours and the biosynthesis of adiponectin was quantified by standard methodology.

Digby JE et al, J Endocrinol 2006

Circulating levels of adiponectin were measured in serum by ELISA
<table>
<thead>
<tr>
<th>Study Population</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>N (males/females)</td>
<td>169 (139/30)</td>
</tr>
<tr>
<td>Age (years)</td>
<td>66.9±0.7</td>
</tr>
<tr>
<td>Hypertension (%)</td>
<td>117</td>
</tr>
<tr>
<td>Smoking (%)</td>
<td></td>
</tr>
<tr>
<td>Active smokers</td>
<td>46</td>
</tr>
<tr>
<td>Ex smokers</td>
<td>70</td>
</tr>
<tr>
<td>Diabetes mellitus (%)</td>
<td>52</td>
</tr>
<tr>
<td>Dyslipidemia (%)</td>
<td>88</td>
</tr>
<tr>
<td>Body mass index (Kg/m$^2$)</td>
<td>27.4±0.3</td>
</tr>
<tr>
<td>Waist:Hip ratio</td>
<td>1.01±0.07</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>T45G</th>
<th>G276T</th>
</tr>
</thead>
<tbody>
<tr>
<td>GG (%)</td>
<td>2 (1.2)</td>
<td>85 (50.3)</td>
</tr>
<tr>
<td>GT (%)</td>
<td>40 (23.7)</td>
<td>68 (40.2)</td>
</tr>
<tr>
<td>TT (%)</td>
<td>127 (75.1)</td>
<td>16 (9.5)</td>
</tr>
</tbody>
</table>
Adiponectin genetic variability & circulating adiponectin levels

Serum adiponectin (μg/mL)

<table>
<thead>
<tr>
<th></th>
<th>TG+GG</th>
<th>TT</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>T45G</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G276T</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

p=NS
Adiponectin genetic variability & adiponectin release from AT

![Graph showing adiponectin levels in AT with genetic variability at T45G and G276T loci.](image-url)

- TG+GG vs TT: *p<0.05
- GG+GT vs TT: **p<0.01

Adiponectin in AT (pg/mg tissue)

**T45G**
- TG+GG
- TT

**G276T**
- GG+GT
- TT
Impact on adiponectin’s biosynthesis locally on AT, but not in the circulating pool

T45G
G276T

Adiponectin

X

?
Could the changes in local adiponectin production affect vascular redox state?
Adiponectin genetic variability & vascular redox state

T45G

Total O2- in IMA (RLU/sec/mg)

<table>
<thead>
<tr>
<th></th>
<th>TG+GG</th>
<th>TT</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* p<0.05

G276T

Total O2- in IMA (RLU/sec/mg)

<table>
<thead>
<tr>
<th></th>
<th>GG</th>
<th>TT+GT</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* p<0.05
Adiponectin genetic variability & vascular redox state

**NADPH O2- in IMA (RLU/sec/mg)**

- **TG+GG**
- **TT**
- **T45G**

- **G276T**
- **GG**
- **TT+GT**

* p<0.05
Study Part B: Is the effect of adiponectin on vascular redox direct?

10 patients with CAD undergoing CABG

CABG

Paired SV segments

Ex-vivo protocol

Control

Adiponectin 10μmol/L

6 hours incubation

Vascular O2- (RLU/sec/mg)

P<0.05

Adiponectin - +
Adiponectin & endothelial function

** p<0.01

FMD (%)

<table>
<thead>
<tr>
<th></th>
<th>T45G</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>TG+GG</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TT</td>
<td>4</td>
<td></td>
</tr>
</tbody>
</table>

**

<table>
<thead>
<tr>
<th></th>
<th>G276T</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>GG+GT</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>TT</td>
<td>2</td>
<td></td>
</tr>
</tbody>
</table>

** p<0.01
Adiponectin & arterial distensibility

**Arterial Distensibility**

(mm x 10^-3 x mmHg)

<table>
<thead>
<tr>
<th>Genotype</th>
<th>TG+GG</th>
<th>TT</th>
<th>GG+GT</th>
<th>TT</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>T45G</strong></td>
<td><img src="#" alt="Bar Chart" /></td>
<td><img src="#" alt="Bar Chart" /></td>
<td><img src="#" alt="Bar Chart" /></td>
<td><img src="#" alt="Bar Chart" /></td>
</tr>
<tr>
<td><strong>G276T</strong></td>
<td><img src="#" alt="Bar Chart" /></td>
<td><img src="#" alt="Bar Chart" /></td>
<td><img src="#" alt="Bar Chart" /></td>
<td><img src="#" alt="Bar Chart" /></td>
</tr>
</tbody>
</table>

* p<0.05
Discussion

Endothelial cell

↑ Adiponectin

↓ O$_2^-$

↑ NO

↓ ONOO$^-$

G276  G45

NADPH oxidase

Arterial Distensibility

Endothelial function

ATHEROGENESIS ?
Conclusions

T45G and G276T polymorphisms on adiponectin gene modify the biosynthesis of adiponectin in human adipose tissue.

These polymorphisms can be used as a model system of chronic hypo- or hyper-adiponectin expression in human adipose tissue.

By using this model system we demonstrated that adiponectin reduces vascular O2- generation via an NADPH-oxidase mediated mechanism in human arteries.

This novel data provides a link between adiponectin release in perivascular adipose tissue and vascular function, defined by arterial distensibility and endothelial function.
These novel findings suggest that adiponectin produced by perivascular adipose tissue may have a direct impact on atherogenesis, providing a new potential therapeutic target in cardiovascular disease.
Exclusion criteria:

1. ACS < 3 months
2. Heart failure (LVEF<40%)
3. Renal / Liver disease
4. Any inflammatory disease
5. Malignancy
6. Treatment with NSAIDs / antioxidants