Serum Insulin & Blood Pressure in Obesity may be Linked to Subcutaneous & Omental Fat ADMA Content

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**Context**

- **Obesity** is an excess of adipose tissue (fat).
- It is the most prevalent nutritional disorder associated with significantly increased risk for **CVD morbidity and mortality**.
- The exact role and mechanisms by which obesity promotes cardiovascular risk is **poorly understood**.
- Obesity is associated with adverse changes in **circulating CVD risk factors**, which may be involved in the development of T2DM and CVD.
Endothelial function determined by bioavailability of NO, and insulin sensitivity modulated by adiposity and altered NO levels, may explain both the endothelial dysfunction and insulin resistance of obesity.

Although it has been postulated that adipose tissue-derived mediators (leptin, TNF-α, IL-6 and adiponectin) act on the endothelium to produce detrimental effects, to date none has been clearly identified.

Asymmetric dimethylarginine (ADMA) - a metabolic by-product of protein modification in the cytoplasm - is an endogenous inhibitor of all forms of nitric oxide synthase (NOS) and is found in plasma.
ADMA inhibition of NO:

\[
\text{L-arginine} + \text{Oxygen} \rightarrow \text{L-citruline} + \text{NO} + \text{Water}
\]

\[
\text{ADMA} + \text{Oxygen} \rightarrow \text{ADMA superoxide radical}
\]

ADMA Inhibits NO Synthesis

![Graph showing ADMA concentration vs. nitric oxide synthesis percentage.](image-url)

Nitric Oxide Synthesis (%)

ADMA Concentration (ng/l)
Effects of ADMA

- Plasma ADMA is raised in CVD, hypertension, atherosclerosis and T2DM.
- ADMA levels are also elevated in morbidly obese subjects.
- Circulating ADMA levels correlate closely with degree of insulin resistance and is independently associated with BMI.
- Significant reduction in systemic ADMA, along with improvement in several components of the metabolic syndrome, follows bariatric surgery and weight loss.

Elevated ADMA Level (Inhibition of NOS)

- Atherosclerosis
- Hypercholesterolaemia
- Liver failure
- Diabetes
- OSA
- Hypertension
- Pre-eclampsia
- CCF
- CVA
- Chronic renal failure
- Erectile dysfunction
- Vascular dementia

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ADMA is synthesized by PRMTs and hydrolysed by DDAH: all components of this pathway are expressed in adipose tissue.
Aims

To investigate the
- depot-specific differences in ADMA content and release, and
- the expression of DDAH 1 & 2 (the enzymes responsible for ADMA hydrolysis) and PRMTs (the enzymes responsible for the synthesis of ADMA) in human omental and subcutaneous adipose tissue.

PRMT: protein arginine methyl transferase; DDAH: dimethylarginine dimethylaminohydrolase;
Method

- A cross-sectional cohort study of *Caucasian morbidly obese*, non-diabetic female patients undergoing gastric banding or cholecystectomy.
- Circulating, adipose tissue content and generation of ADMA and tissue expression of DDAH-1 and -2 mRNA, and PRMT-3 protein were determined from omental and subcutaneous depots.
- In a subgroup of patients (n=9) the stroma-vascular fraction was separated from whole adipose tissue and ADMA and DDAH were analysed.
- Insulin resistance was assessed by HOMA-IR and body fat content by electrical bio-impedance.
- Exclusion: DM, CHD, HTN, conditions or agents affecting cytokine release e.g: aspirin, NSAIDs, steroids, warfarin, ACE inhibitors, statins

HOMA-IR: Homeostatic Model Assessment-Insulin Resistance
\[ \text{HOMA-IR} = \frac{\text{fasting plasma insulin (\(\mu\)IU/mL)} \times \text{fasting plasma glucose (mmol/L)}}{22.5} \]
Schema of Methods

Anthropometric Measurements (Body stat analyser)
- BMI
- % Body Fat
- % lean Mass

Fasting Blood
- Lipids
- Glucose
- Insulin
- ADMA

HOMA-IR

Fat biopsy at surgery

Tissue ADMA
- Organ Culture ADMA
- Stroma-Vascular Fraction (SVF) separated

HPLC: High performance liquid chromatography

mRNA for DDAH
- Protein for PRMT

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## Anthropometric and Metabolic Characteristics of Patients

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
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<tbody>
<tr>
<td>Number</td>
<td>16</td>
</tr>
<tr>
<td>Age (years)</td>
<td>43.4 (7.9)</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>43.8 (10.1)</td>
</tr>
<tr>
<td>Body fat (%)</td>
<td>52.6 (7.0)</td>
</tr>
<tr>
<td>Lean mass (%)</td>
<td>47.4 (7.0)</td>
</tr>
<tr>
<td>Insulin (MU/l)</td>
<td>7.7 (7.1 - 15.0)</td>
</tr>
<tr>
<td>Glucose</td>
<td>5.18 (0.45)</td>
</tr>
<tr>
<td>Total Cholesterol (mmol/l)</td>
<td>4.56 (0.93)</td>
</tr>
<tr>
<td>HDL cholesterol (mmol/l)</td>
<td>1.21 (0.24)</td>
</tr>
<tr>
<td>LDL cholesterol (mmol/l)</td>
<td>2.8 (0.92)</td>
</tr>
<tr>
<td>Triglycerides (mmol/l)</td>
<td>1.12 (0.32)</td>
</tr>
<tr>
<td>ADMA (mM)</td>
<td>1.95 (1.05 - 2.06)</td>
</tr>
<tr>
<td>Adiponectin (mg/ml)</td>
<td>5.6 (2.2 - 13.3)</td>
</tr>
<tr>
<td>Leptin (ng/ml)</td>
<td>28.7 (18.2 - 40.7)</td>
</tr>
<tr>
<td>IL-6 (pg/ml)</td>
<td>2.22 (1.38 - 2.39)</td>
</tr>
<tr>
<td>MCP-1 (pg/ml)</td>
<td>221.3 (173.0 - 244.4)</td>
</tr>
<tr>
<td>RANTES (ng/ml)</td>
<td>57.0 (35.1 - 66.9)</td>
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Results 1

- Serum insulin and systolic blood pressure correlated directly with subcutaneous ADMA content.
- ADMA release was significantly higher from the omental depot (p=0.025) and correlated with BMI.
- While DDAH2 expression was higher compared to DDAH1 in both the whole adipose tissue and the stroma-vascular fraction of both depots.
- No depot-specific difference in the expression of either isoform was detected.
- However, PRMT-3 protein expression was higher in the omental compared to the sub-cutaneous adipose tissue.
ADMA Release

- Omental vs. Subcutaneous
- Box plot showing ADMA release (μM/g tissue/24h)
- Comparison by Wilcoxon Rank test
- p = 0.025

n=13
DDAH Expression - Adipose Tissue

$n=15$, Comparisons by Wilcoxon Rank test
DDAH Expression - Adipose Tissue vs SVF

n= 9, Comparisons by Wilcoxon Rank test

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Results 2

- Om ADMA release \( \alpha \) BMI \((r = 0.56, p = 0.04)\)

- HOMA-IR \( \alpha \) Sc tissue ADMA \((r = 0.83, p = 0.04)\)
  Om tissue ADMA \((r = 0.70, p = 0.08)\)

OM: Omental; SC: Subcutaneous; HOMA-IR: Homeostatic Model Assessment-Insulin Resistance
Conclusion

- The direct associations of omental ADMA release and BMI and higher omental ADMA content points to a link between visceral obesity and endothelial dysfunction.
- The depot-specific generation in ADMA may be due to differences in the synthetic enzymes or to changes in the activity, rather than mRNA expression, of DDAH.
- Modulation of adipose ADMA generation may reduce obesity associated co-morbidities.