The role of oxygen insufficiency in the onset and development of the vascular complications of diabetes

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Where?
Oxygen and cell survival

– Capillaries bring oxygen and nutrients to cells
Glucose concentration regulated by insulin
Unregulated glucose level

- Diabetes mellitus (DM)
  - Type 1 DM – insulin insufficiency
  - Type 2 DM – insulin resistance

- Clinical management of glucose levels is a priority

- High and/or fluctuating high levels of glucose increase the vascular complications resulting from the disease.
Glucose mediated endothelial cell damage

- Oxidative stress
- ↓ cell proliferation
- ↑ activation/adhesion
- Impaired O2 delivery
- Anaerobic glucose metabolism
Hyperglycemia

↑ DAG

↑ PKC
(β- and δ- isoforms)

↓ eNOS  ↑ ET-1  ↑ VEGF  ↑ TGF- β  ↓ Collagen  ↑ Fibronectin  ↑ PAI-1  ↑ NF-κB  ↑ NAD(P)H oxidases

Blood-flow abnormalities  Vascular permeability Angiogenesis  Capillary occlusion  Vascular occlusion  Pro-inflammatory gene expression  Multiple effects
Anaerobic vs aerobic metabolism

Robert A. Gatenby & Robert J. Gillies
Oxygen insufficiency

oxygen supply  oxygen demand

oxygen supply  oxygen demand

Adaptive response
<table>
<thead>
<tr>
<th>Consequence of normoglycaemia and low oxygen?</th>
<th>Rationale for the adaptive response</th>
<th>Consequence in hyperglycaemia and low oxygen?</th>
</tr>
</thead>
<tbody>
<tr>
<td>&gt; anaerobic metabolism therefore reduced ATP production</td>
<td>to increased glucose uptake to redress balance and increase ATP production</td>
<td>glucose levels may be already very high and increase oxidative stress</td>
</tr>
<tr>
<td>&gt; glucose metabolism</td>
<td>to increased glucose metabolism to redress balance and increase ATP production</td>
<td></td>
</tr>
<tr>
<td>&gt; endothelial cell proliferation</td>
<td>to increase vascular areas and thus enhance oxygen delivery</td>
<td>hyperglycaemia modifies some of the structures within cells that are able to respond to mediate cell proliferation reducing ability of cells to proliferate</td>
</tr>
<tr>
<td>&gt; erythropoietin production</td>
<td>to increase oxygen delivery to the cells/tissue</td>
<td></td>
</tr>
</tbody>
</table>
Endothelial cells

3D projection of a confocal z-stack shows human umbilical vein endothelial cells (HUVECs) forming a functional vessel immunofluorescently stained for PECAM-1 (green) and nuclei (blue). (Wong/Searson Lab)
Capillary damage/dysfunction

- Oxygen insufficiency
  - Hypoxia inducible factor type 1 (HIF1)
  - Dimeric transcription factor
    - HIF1α – stabilised in oxygen insufficiency
    - HIF1β – constitutively expressed
- Binds to hypoxia response element (HRE)
Hypoxia response element

HRE

-947

5' C C A C A G T G C A

-939

3' G G T G T C A C G T

Match

HRE

3' A T G C A C C C G A G

Mismatch

FITC

+ NH₃

1

FITC

+ NH₃

2
Hypoxia inducible factor type 1

Angiogenesis
- EG-VEGF
- ENG
- LEP
- LRP1
- TGF-β3
- VEGF
- VEGFR
- ADM
- ET1
- α1b-AR
- HO1
- NOS2

Growth & Survival
- Cyclin G2
- IGF-BP1, 2, 3
- WAF-1
- TGF-α
- TGF-β3
- ADM
- EPO
- NOS2
- NIP3
- NIX
- RTP801
- ET1
- VEGF
- VEGFR
- Transferrin
- Transferrin-R
- MDR

Glucose metabolism
- HK1
- HK2
- AMF/GPI
- ENO1
- GLUT1
- GLUT3
- GAPDH
- LDHA
- PFKBF3
- PFKL
- PGK1
- PKM
- TPI
- ALDA
- ALDC
- LEP

Invasion & Metastasis
- KRT14
- KRT18
- KRT19
- VIM
- MIC2
- CATHD
- Collagen type V (α1)
- FN1
- MMP2
- PAI1
- Prolyl-4-hydroxylase (α1)
- UPAR
- AMF
- c-MET
- LRP1
- TGF-α

Miscellaneous
- DEC1, 2
- ETS-1
- NUR77
- CA 9
- p35srj
- ITF
- AK3
- ECTO-5'-nucleotidase
- Ceruloplasmin
- Transglutaminase 2
Glucose mediated change to HIF1 function

HIF1\(\alpha\) p300

High glucose induced conformational change of p300

Activation of transcriptional activity

HIF-1\(\alpha\) dysfunction in diabetes Cell Cycle 9:1, 75-79; January 1, 2010; Hariharan Thangarajah, Ivan N. Vial, et al.
Human microvascular dermal endothelial cell model
Image taken each day and stored for subsequent analysis
Effect of [glucose] and [oxygen]

Summary

Hypoxia increased net migration distance at 24 & 48 h.

High glucose concentration decreased net migration of endothelial cells at 24 & 48 hours.
Human microvascular dermal endothelial cell (HMVDEC) model

Normoxia (18% oxygen)

Hypoxia (5% oxygen)

5mM glucose

20mM glucose
Image taken each day and stored for subsequent analysis
Effect of mannitol

Summary

Hypoxia increased net migration distance at 24 & 48 h.

Increased mannitol concentration did not show any significant change in the net migration of endothelial cells at 24 & 48 hours.
**Effect of wounding cells**

**Summary**
Hypoxia increased migration and an increased glucose concentration decreased the net migration. Cells from the wounded edge travelling at a significantly greater distance than cells from intact edge.
Immunostaining: HIF1α

Hector, MacMannus & Knott (2004)
Immunostaining: HIF1α

Intact edge

Wounded edge

5 mM glucose hypoxia

20 mM glucose hypoxia

HuMVDEC
Reactive oxygen species

- **Silymarin**
  - added in liquid form

**Summary**
Silymarin restores glucose mediated decreased cell migration.
• **Silymarin**
  – Formulated with lyophilised wafers for topical application

**Summary**
Silymarin can be incorporated into a lyophilised wafer for topical application to recalcitrant wounds.
Retinal explant model

Agarose and collagen retina

Agarose and collagen

Agarose + Collagen

Agarose
Haematoxylin and eosin staining of retinal explant

- Model shows retinal structural changes

Choroidal layer

Vitreous layer

Scale 100um: 40X

0 HOURS  24 HOURS  96 HOURS
Immunohistochemistry (HIF1α) of retinal explant
Hyperbaric oxygen therapy (HBOT) is done in a sealed chamber pressurized at 1 ½ to 3 times normal atmospheric pressure where the patient is breathing pure oxygen.
Treatment of recalcitrant ulcers
Mechanism of action

- O2 carried by erythrocytes
- HBOT increases O2 solubility
- Crosses cell membranes entering bodily fluids
  - Plasma, lymphatic system, interstitial fluid, cerebrospinal fluid
- Toxicity
  - Lungs (oedema)
  - CNS toxicity (grand mal)
  - Eyes (myopia)
Capillary growth - angiogenesis

- Oxygen under pressure forces more oxygen into the tissues and encourages new blood vessels to grow.
- Plasma can carry 100% oxygen under pressure.
- Extra oxygen is diffused to the tissues from the new blood vessels.
- Red blood cells flow into new blood vessels.
- Diffused oxygen.
HBOT and hyperoxia
Treatment of recalcitrant ulcers
• Summary

– Greater understanding of mechanisms of vascular disease
– Facilitates development in management of diabetes
– Provides opportunities for continuing development of therapeutic options