Vascular Biology and Haematology

Lecture 1: Introduction
Functions of Blood:

1. Transport of: Gases:
- Oxygen between the lungs and tissues for respiration and metabolism.
- CO2 between the tissues and the lungs for removal form the body
- Nutrients - carbohydrates, amino acids, fats, protein from gut to tissues.
- Hormones
- Removal of waste products: From tissues to liver and kidneys for processing and excretion.

2. Regulations of Fluid, pH and Temperature
3. Protection of the immune system.

Therefore, it is vital that the body has a proper functioning vascular system to carry out these vital functions.

Key components of the vascular system:
Cells:

- RBC: carry O_2/CO_2 (although most is carried as bicarbonate in the plasma)
- Platelets: Maintains vascular integrity
- WBC: Immune cells.

Liquid:

- Plasma - Over 90% is water with remaining 10% being mostly protein with some electrolytes, vitamins and nutrients such as glucose and amino acids.

Pump and the piping

- Heart: pumps blood
- Blood vessels - arteries, arterioles, capillaries, venules, veins: These are vessels that carry the blood to all parts of the body. This allows for perfusion to all tissue beds and cells.
- Lymphatics: drainage of tissue fluid back to the blood.

Blood Cell Types and Numbers

Normal Ranges of Blood Cell Counts for Healthy Adults and Children

	Red Cells per microliter (µL) of blood	White Cells per microliter (µL) of blood	Platelets per microliter (µL) of blood	Hematocrit[1] % of blood composed of red cells	Hemoglobin[1] grams per deciliter (g/dL)
Men	4.7 to 6.1 million	5,000 to 10,000	150,000 to 400,000	42 to 52	14 to 18
Women[2]	4.2 to 5.4 million	4,500 to 11,000	150,000 to 400,000	37 to 47	12 to 16
Children[3]	4.0 to 5.5 million	5,000 to 10,000	150,000 to 400,000	32 to 44	9.5 to 15.5

RBC do regulate their Hb concentration and volume.

Blood Cell Adhesion
RBC:

- Physiological: do not normally adhere, they are transporters that flow through the vessels without sticking.
- Pathological: adheres in some pathological situations

Leukocytes:
They have to adhere to get into the tissue. It is important for inflammatory responses. During chronic infection, WBC will adhere more due to the inflammatory cytokines that were release to accelerate the accumulation of more WBC.

- Physiological: protective inflammatory response, Lymphocyte re-circulation
- Pathological: out of control/non-stop, Vascular Occlusion, tissue damage.

Platelets:

- Physiological: haemostasis (e.g. damaged vessel)
- Pathological: thrombosis - occlusion.

Blunting of velocity profile (plug flow):

- Velocity profile is blunted at haematocrits over 30%
- This occurs at both low and high flow rates
- WBC and platelets move towards the edge of the flow.
- Margination: RBC pulls into the centre of the vessel and the aggregation of the RBC pushes the WBC out to the side of the cells. This is important for the movement of the WBC into the tissues
    - In venules, the cells can overcome the force, and stick to the walls and go into the tissues.

Leukocyte migration:
This process is stimulated by the presence of cytokines or infection -

- Margination allows the WBC to come in contact with the cell wall.
- The WBC starts to slow down and roll of the vessel wall surface.
- There are selectins on the endothelial wall that weakly interact with the proteins on the leukocyte.
- Binding to selectins, sends activating signals from the endothelium, so that firm adhesion of the leukocyte can occur.
- The activating signals allow the leukocytes to spread, so they become elongated and start to change their shape.
- Leukocytes then migrate over the top of the endothelial surface and eventually migrate through the endothelial cells into the tissues down the gradient of cytokines

Leukocyte migration: Problems to solve

- Catching a fast moving leukocyte
- Stabilising adhesion
- Present activation agents e.g. chemokines

Lecture 6: Haemostasis: Coagulation and Platelet Function Testing

Coagulation Cascade: Generation of Fibrin.

Haemostasis is a multistage process:
Step 1: Blood Vessel Response – Vasoconstriction reduces the blood loss at the site of injury
Step 2: Activation of platelets and formation of platelet plug
Step3: Blood Coagulation – Intrinsic eland Extrinsic Pathways
Step 4: Clot Retraction and Fibrinolysis

Thrombin is a key molecule. It can set off the events that are required. It is converting the plasma protein fibrinogen into fibrin, which helps to reinforce the aggregate.

Initiation stage of coagulation - Extrinsic Pathway:
All the compounds that are important in the generation of thrombin and fibrin in the clots.
Extrinsic pathway is the spark to generate small amount of thrombin. This is a biochemical pathway.
There are a series of factors (FI-FXIII) which are involved in the generation of fibrin.

Extrinsic Pathway (initiation coagulation):

- Starts with the exposure of subendothelial proteins that are not usually present in the blood.
- Tissue Factor (TF) (Factor III) is exposed when damage occurs to the endothelial.
- TF is able to bind to the circulation blood protein, Factor VII. The binding of TF to FVII causes the activation of FVII, giving FVIIa.
- FVIIa is able to activate Factor X (FX), in the presence of Ca2+, to give FXa.
- FXa is essential, and in combination with FVa, Ca2+ and Phosphatidyl serine, it allows the conversion of prothrombin to thrombin.

Amplification stage of coagulation - Intrinsic Pathway:
Intrinsic pathway allows for generation of small amounts of thrombin. It is a spark. Once the initial thrombin is generated, we need more. All the factors are present in the blood, but they just need to be activated to start the process off.

- Factor XII is activated by tissue damage. This gives FXIIa.
- FXIIa is able to activate Factor XI to give FXIa
- FXIa activates Factor IX to give FIXa.
- FIXa alongside FVIIIa, Ca2+ and Phosphatidyl Serine allows the activation of Factor X (FX)
- FXa is essential, and in combination with FVa, Ca2+ and Phosphatidyl serine, it allows the conversion of prothrombin to thrombin.

Factor XII activates the intrinsic pathway, but it is not essential. It can be removed and this pathway can still be generated. Taking away the other factors will result in haemophilia. This is because the major role is for the amplification of thrombin to carry out its role. Thrombin itself can activate this pathway so small amounts of thrombin can generate large amounts of thrombin. Thrombin can also generate FVIIa, FVa which are needed

Thrombin reinforces its own production. it helps to drive its own generation.

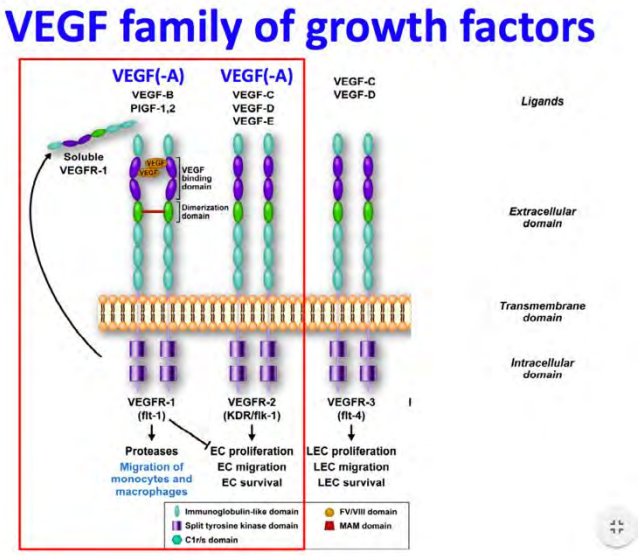
End Result of Coagulation - Fibrin and the common pathway:

- Increased plasminogen activator inhibitor (PAI)-1 expression
- Von Willebran factor (vWF) released from Weibel Palade bodies

When there is samage to the endothelium, under the endothelial cells, there are collagen in the basal lamina. Platelets gets activated by collagen, causing the platelets to bind and become aggregated and activate. The endothelial cells will also dump Von Willebrand factor which sits in the Weibel Palade bodies and they also encourage platelet aggregation and activation.
It will decrease the levels of the things that activate fibrotic agents such as plasmin so it will keep the clots that are forming in place. Damage inflammation will up regulate tissue factor and it can be made by large amounts of the tissue endothelial cells and will then trigger coagulation clotting. This goes from low to high levels very rapidly since thrombin helps with its own production.

Vascular Endothelial Growth Factor

- VEGF is the most significant member of a family of growth factors
  VEGF (-A), B, C, D and Placenta growth factor/PIGF
- A master regulatory of vasculogenesis (process of forming endothelial cells and haematopoietic cells), physiological and pathological angiogenesis
- Essential for maintenance of vascular homeostasis (maintains vasculature in a healthy state)/ EC survival in the adult
- Pleiotropic effects on selectivity on endothelial cells via two high affinity receptors.



Activity is governed by the distribution of receptors.
There are 2 key tyrosine receptors for VEGF-A and 3rd tyrosine kinase receptor which becomes located on the lymph endothelium and through VEGF C and D, it drives the lymphatics for the survival of the lymphatics.

VEGF-A binds to VEGFR-2 (KDR/flik-1). This is on the haematopoietic and angioblast progenitors that is present and causes signalling. It causes EC proliferation, migration and survival.

VEGF-1 (flt-1) is another receptor that will detected later. In the endothelium it is more of a negative regulator and keeps the activity of VEGF in check and it is also made in a soluble form the circulates. It has very high affinity for VEGF. This soluble form can bind to VEGF in the vasculature and can regulate its activity. Once VEGF is bound to it, VEGF cannot bind with its receptors so it cannot signal into the endothelial cells. The receptor is also present on inflammatory cells, and VEGF plays an important role in inflammatory conditions and regulating them.

VEGF Expression is tightly regulated since VEGF levels must be regulated within a narrow concentration range for development and vascular homeostasis

Hormonal Influences on Blood Vessels:
NA: α1/ α2 receptors. α1 go through the IsnP3 pathway and result in the opening of VG Ca2+ Channels. α2 link to the K+ channels, closing K+ channels, therefore increases depolarisation of the cell, leading to calcium influx into the cell. Both α1/ α2 receptors increase Ca2+ concentrations in the cell and they result in the release of NO from the endothelial call and so this limits the contraction of it's own effects on the VSM.
Adrenaline: β2 receptors. Adrenalin binds to receptor, and this triggers the NO pathway in the endothelial cell. This also acts on the VSM, to increase cAMP in the muscle, cAMP binds to MLCK and inhibits it, so the VSM undergoes relaxation.
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Vasopressin and Angiotensin: VP binds to V1 receptors and ANG binds to AT1 receptor. Both are present on the VSM.
link into the IsnP3 pathway - they both release Ca2+ from the sarcoplasmic reticulum causing vasoconstriction

In the brain, there are tight endothelial junctions and basement membrane (form the blood brain barrier), which prevent diffusion of hormones to VSM. It can only act on the brain when the blood brain barrier is broken/ damaged.

Local (humoral) influences on blood vessels "Flow-Metabolism Coupling":
Described how blood flow to a tissue is matched to its O2 requirements and the mechanisms underlying this.

- Tissues generally metabolise by oxidative metabolism
- Tissues vary in their resting metabolism and in the extent to which metabolism is increased or decreased under different circumstances.
- Normally, blood flow changes directly with metabolism and this relationship depends on local mechanism. If metabolism increases so does blood flow, this occurs by dilation.
- Indicated that substances, which are released locally in proportion to metabolism, causes dilation of blood vessels - arterioles. So there are there is something from the increase in metabolism which causes dilation of arterioles. A number of different substance's that do this e.g. NO
- The substance/substances responsible vary between different tissues.
  Partly explained by special characteristics of tissue cells
  Also reflects different characteristics of VSM and endothelium in different tissues. Sometimes, the substances that are released by the tissues cells acts directly on the VSM, and so sometimes they act via the endothelium.
  In some cases, there are things that are released by RBC that act on the endothelium to help increase in blood flow which an increase in metabolism.

Flow-Metabolism Coupling in different tissues:
Skin - Low resting metabolism and tissues O2 consumption (VO2)

- Little variation in different conditions.

Kidney - Has a high resting metabolism (due to all the pumps and transport mechanisms) and has a high tissue VO2 (Oxygen usage)

- But very little variation under different conditions.

Skeletal Muscle - Low resting metabolism and tissue VO2

- Large scope for increases in metabolism and in VO2 in exercise

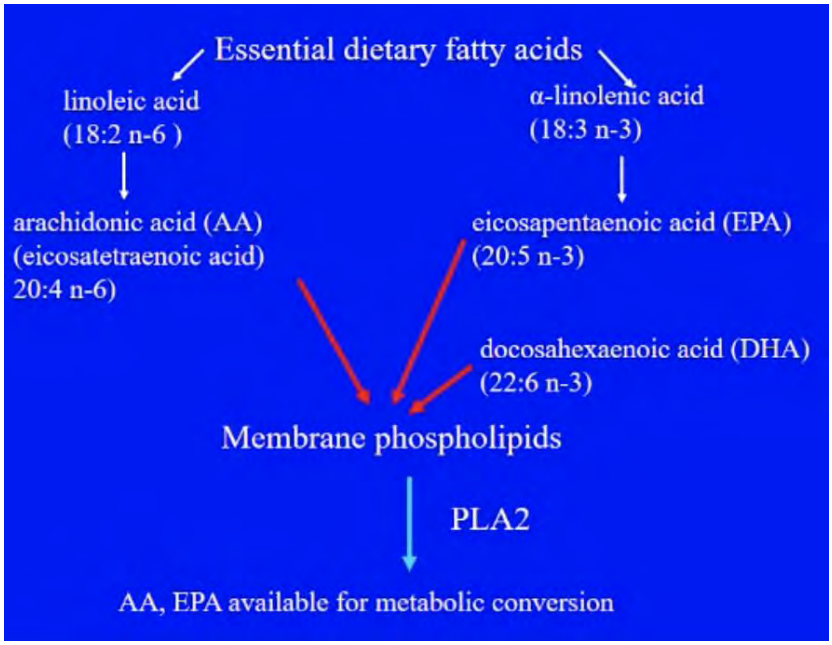
Cardiac Muscle - High 'resting' metabolism and tissue VO2

- Large scope for further increases when cardiac work is increased - this is accompanied by an increase in blood flow

- Femoral Artery Bypass Surgery - same procedure and bypass blockages in the femoral arteries using vein grafts
- Carotid Enterectomy - unblock the bifurcation of the carotid artery. Artery is opened and the plaque is removed
- Angioplasty - Placing a catheter with a balloon in the disease coronary artery. Once the balloon is in place, it is inflated and moves backwards and forwards, removing the plaque.
- Stenting - The balloon goes into the artery and it is inflated and outside the catheter there is a metal stent. This stent expands and which drives into the artery wall, compressing the plaque. It widens the lumen of the artery.

Omega-3 Fatty Acids
Found in fish. They have anti-inflammatory and cardioprotective properties. The two that are biologically active are EPA (Eicosapentaenoic Acid) and DHA (Docosahexaenoic Acid)

Omega-3's can be synthesized in cells but we need essential dietary fatty acids. These are linoleic acid, which are pre-cursors for arachidonic acid (AA). There is also alpha-linolenic acid, which is a precursor for EPA. There is also DHA. All AA, EPA and DHA are converted and stored in the membrane phospholipids. When the cell becomes activated, enzymes such as PLA2 (phospholipase A2), remove the EPA and AA out of the membrane for metabolic conversion



Metabolism of EPA and DHA from dietary ALA is very inefficient
35% of ALA is metabolised for energy. By the time you get to EPA - only 8% of radiolabelled ALA in men and 22% of radiolabelled ALA in woman. By the time you get to DHA - for men, it was undetectable and for female, 9%. This means metabolism is inefficient

Dietary Uptake is Efficient
Formation of omega-3's polyunsaturated acids occur in phytoplankton in the sea. The phytoplankton have the ability to generate the omega 3. Then they get eaten and the predator animals concentrates the omega 3's in their flesh.

Body can efficiently take by EPA from the body when supplementing with Omega-3s. After 24 hours of supplementing, the endothelial cells take up EPA from the baseline which is 2%, to 7-8% of the fatty acids in the phospholipids in those are EPA.

- Sprouts are coming from different vessels, growing to one another
- The two sprouts come together and fuse.
- There is signaling between the endothelial cells and pericytes to maintain quiescence of newly formed vessels, through Angiopoietin 1.
- There is formation of tight junctions and barrier function
- There is also new basement membrane deposition
- There is also pericyte maturation due to the presence of Ang1.
- The area that is was hypoxic is no longer hypoxic and so the stimulus e.g. VEGF for angiogenesis will now be gone.
- The downregulation of the signal and re-establishment of the quiescent vessel is important for the end of angiogenesis.

Exercise and angiogenesis in skeletal muscle:

- Exercise and the contractile activity of muscle influences capillary growth
- Mechanical stretch and shear stress (flow) stimulate angiogenesis
- VEGF plays an essential role in driving capillary growth
- Shear stress primarily induces longitudinal splitting (Intussusception), and this require eNOS signaling
- Tissue stretch involved in muscle contraction induces angiogenic sprouting

Pathologic Angiogenesis

- Angiogenesis contributes to the pathology of inflammatory diseases and the growth and metastasis of solid tumours
- Tumour growth and hyperplasia in inflammatory disease increases the distance of cells from vessels and hypoxia drives VEGF production
- Chronic hypoxia giving rise to high levels of VEGF and inflammatory cytokines create a pro-angiogenic environment. Vessel formation is abnormal leading to leaky and poorly perfused vessels and persistent hypoxia. There is no single direction of the blood flow or clear hierarchy. The leaky vessels facilitate leukocyte extravasation in inflammatory disease

Synovial Hyperplasia results in increased demand of oxygen and nutrients and increased distance from blood vessels. This leads to hypoxia and angiogenesis can occur. There is an increased number of synovia vessels and there is synovia infiltration of cytokines and growth factors. This drives the synovia hyperplasia, and this contributes to angiogenesis.

Modulating angiogenesis – inhibiting VEGF signaling:

Monoclonal antibody to VEGF

- Bevacizumab / Avastin – colon cancer
- Treatment of cancer and diabetic retinopathy

Soluble receptor

- VEGF trap / aflibercept

Small molecule inhibitors of VEGFR – stope the tyrosine kinase domain from functioning. They are not as specific since they affect similar receptors.

- Sunitinib / sutent, sorafenib / nexavar
- Cancer treatment

Antibody blocking binding of VEGF to receptor

- Ramucirumab (IMC 1C11)