Elevated cholesterol levels skew monocytes towards an inflammatory (M1) profile, potentially increasing CVD risk

Jasmin Huang[1,2], Habib Francis[1,2], Rekha Marimuthu[1,2], Heather Medbury[1,2], Stephen Li[3], Rana Baraz[1] and Helen Williams[1,2]

1. Westmead Hospital, Department of Surgery, Westmead, NSW, Australia
2. The University of Sydney, Sydney Medical School, Westmead, NSW, Australia
3. Institute of Clinical Pathology and Medical Research, Westmead, NSW, Australia

Background:

Atherosclerosis, the underlying cause of cardiovascular disease (CVD), is characterised by the build-up of plaques in the arteries. The monocyte, a type of white blood cell plays a crucial role in plaque stability.

Cholesterol is also an important contributor, but the influence of lipids on monocyte inflammatory status has yet to be clearly defined. This is relevant in determining whether people with high cholesterol could benefit from additional treatment targets. Here we aim to investigate whether elevated lipid levels promote phenotypic alterations in hypercholesterolemic patients using specific M1 (pro-inflammatory) and M2 (anti-inflammatory) surface markers, and whether this differs with causation-familial hypercholesterolemia (FH) or non-familial hypercholesterolemia (NFH).

CVD #1

Cause of death globally

1 in 4 deaths

in Australia due to CVD

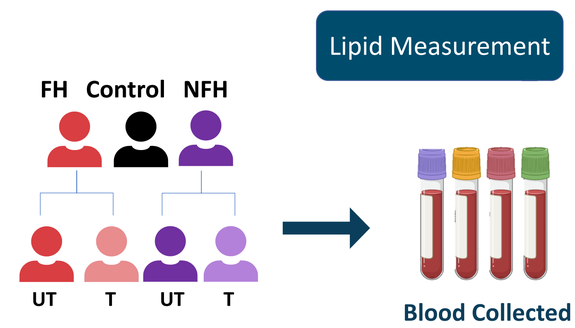
Kills one Australian every **12mins**

Aims:

1) Determine the phenotypic profile of monocytes in hypercholesterolemic patients according to their M1 and M2 surface markers, and whether these characteristics resolve in treated patients
2) Investigate correlations of LDL levels with M1 and M2 surface marker ratio
3) Examine whether monocyte phenotype influences macrophage polarisation

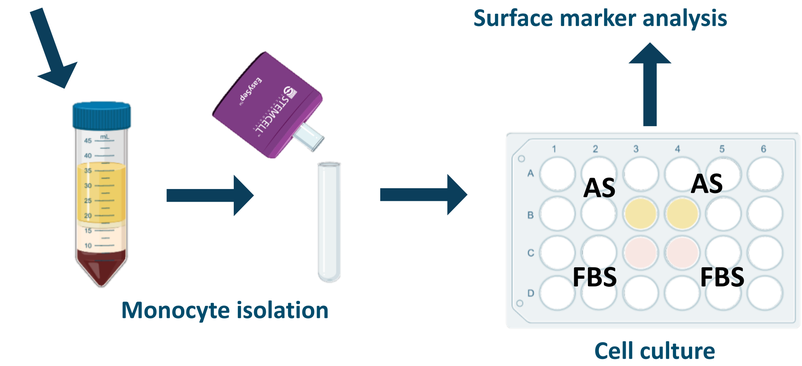
Methods:

Blood was collected from hypercholesterolemic patients (FH and NFH) and controls, with both FH and NFH groups further categorised into treated (T) and untreated (UT).



Surface marker expression (M1 and M2) on monocytes were analysed directly using whole blood flow cytometry, and relative expression was determined as the shift against the isotype control.

Analysis of monocyte-derived macrophages: cells were isolated via density gradient centrifugation and with negative immunomagnetic selection. Cells were cultured for 7 days in either Fetal Bovine Serum or autologous serum. Cells surface markers M1 (CD86) and M2 (CD163) were analysed with flow cytometry.



Hypothesis:

➢ **Untreated**

Untreated FH / **Untreated NFH** → Elevated LDL cholesterol → Elevated M1:M2 surface markers on monocytes → Inflammatory macrophages

➢ **Treated**

Treated FH / **Treated NFH** → Reduced LDL cholesterol → Elevated M1:M2 markers on monocytes → Inflammatory macrophage

Reduced LDL cholesterol → Reduced M1:M2 markers on monocytes → Reduced inflammatory macrophage

Results:

Table 1. Cholesterol levels across the different groups measured as Mean±SEM
* P<0.05 vs. controls, † P<0.01 vs. controls

Participant Groups	Cholesterol (mM)	LDL (mM)	HDL (mM)	ApoA1 (g/L)	ApoB1 (g/L)
Control *n=9*	4.2±0.82	2.2±0.21	1.5±0.15	1.5±0.7	0.7±0.06
FH:(Untreated) *n=6*	8.8±02.0†	6.3±0.82†	1.8±0.09	1.8±0.1	1.6±0.17†
FH: (Treated) *n=8*	5.8±0.96	3.6±0.42	1.8±0.14	1.7±0.12	1±0.08*
NFH:(Untreated) *n=6*	7±0.28†	4.7±0.38†	1.7±0.19	4.4±0.58	1.6±0.12
NFH: (Treated) *n=4*	4.9±0.52	3±0.33	1±0.15	5.1±0.74	1.3±0.07

1) Phenotype of monocytes remains inflammatory following lipid-lowering treatment

Using flow cytometry, the degree of M1 and M2 cell surface markers were analysed.

- Both patient groups (FH and NFH) had an increased degree of expression of M1/M2 ratio.
- This was at a significant level in FH patients and persisted in treated patient groups.

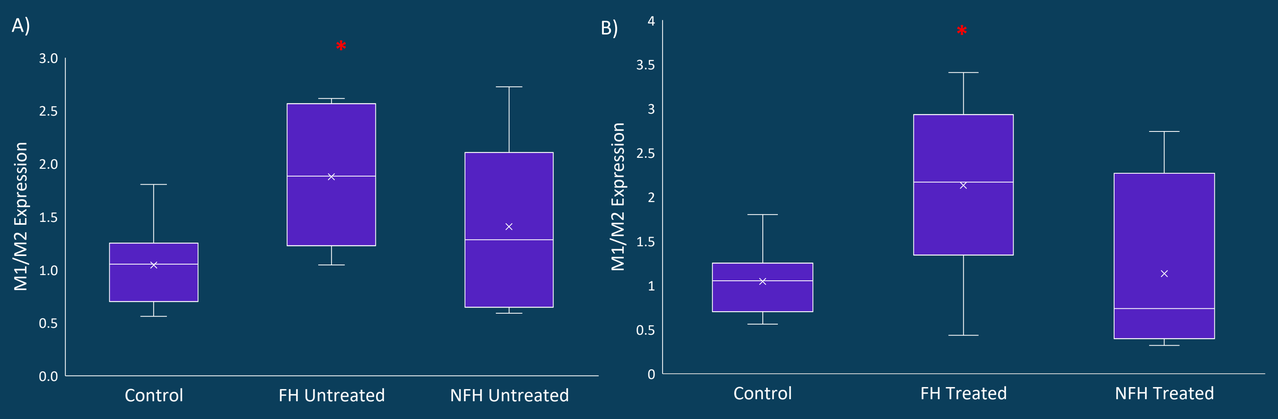


Fig 1. Degree of CD86(M1)/CD163(M2) marker ratio on monocytes in A) **untreated** and B) **treated** FH and NFH groups
* P<0.05 vs. controls

2) M1:M2 ratio is positively correlated to LDL levels in FH patients

- This ratio was correlated with LDL and ApoB levels in all study participants

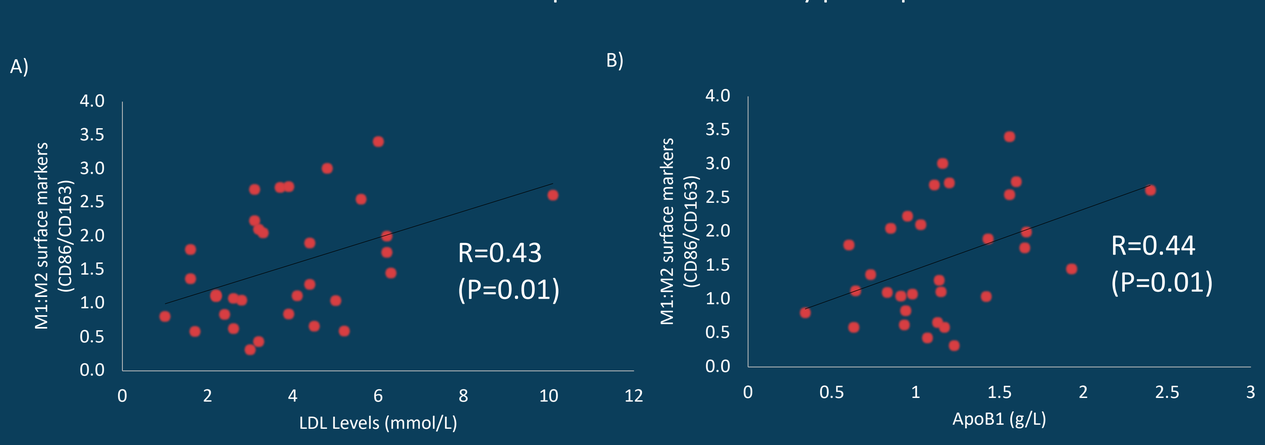
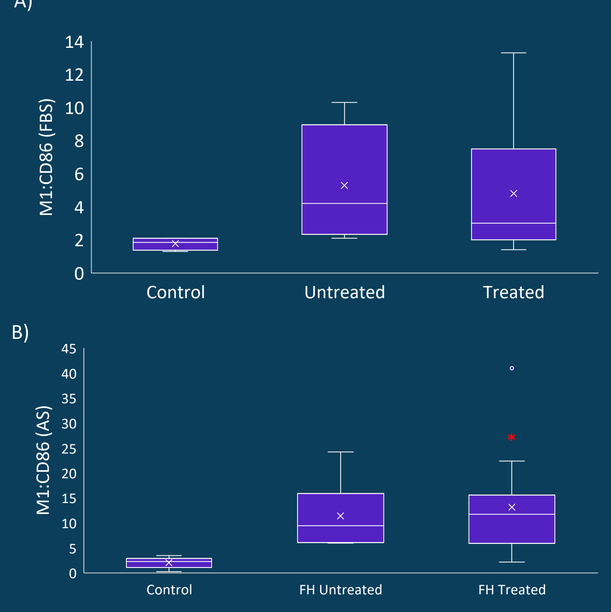


Fig 2. Correlation of M1/M2 surface marker ratio on monocytes in all participants to A) LDL and B) ApoB

3) Inflammatory monocytes influence macrophage phenotype

- Monocytes cultured in autologous serum retained elevated levels of M1:M2 marker expression in pre- and post- treated FH patients



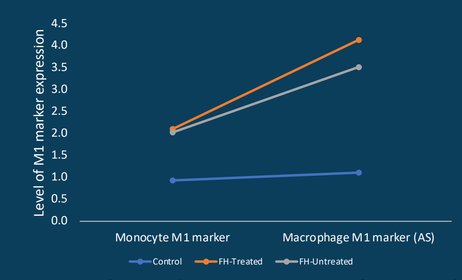


Fig 4. Correlation between M1 surface markers on monocytes and macrophages across target groups

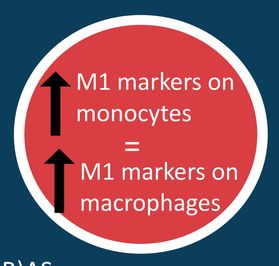


Fig 3. Degree of M1 marker expression on macrophages cultured in A)FBS and B)AS
* P<0.05 vs. controls

Discussion/ Conclusion:

- These results indicate a positive correlation of elevated cholesterol levels with an inflammatory monocyte phenotype, and the resulting macrophage retain this pro-inflammatory phenotype.
- As such, high cholesterol level is likely to also contribute to CVD inflammatory risk factors.
- Current cornerstone treatment with lipid lowering medication does not address monocyte alteration, highlighting the potential importance of anti-inflammatory treatment in management of CVD.