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# Scientific Literature Review



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# Overview:

1. **What is a Scientific Literature Review?**
2. **How to write a Scientific Literature Review**
3. **Key elements of a Coherent Literature Review**
4. **Literature Review Structure**



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# What is a Scientific Literature Review?



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# Scientific Literature Review:

A scientific literature review is a **critical** account of what has been published on a topic by accredited researchers.

It may be:

- A stand-alone assignment
- An introduction to an essay, report, thesis, etc.
- Part of research/grant proposals



# Scientific Literature Review:

## Writing a literature review will:

- Improve your topic knowledge
- Provide new insight on your topic to others
- Demonstrate your literature searching abilities
- Demonstrate your critical analysis skills
- Demonstrate your communication/writing skills

***...your lecturer will be marking you on these skills!***



# Scientific Literature Review:

A scientific literature review is **not**:

- An English essay... use *scientific writing*!
- A summary of each research article that you read
- Based on personal opinion or biased towards your opinion
- A chronological history of events in your research area



# Scientific Literature Review:

What is the purpose of a literature review?





# Scientific Literature Review:

## What is the purpose of a literature review?

*Communication and advancement of scientific knowledge!*

- Scientific knowledge is **not static**: reviews help scientists to understand how knowledge in a particular field is **changing and developing** over time
- There is a **significant output** of scientific publications – literature reviews save time for the scientific community
- Literature reviews can lead to **new scientific insights** and highlight gaps, conflicting results and under-examined areas of research



# Scientific Literature Review:

## A scientific literature review should:

- Provide a **clear statement** of the topical area (scope)
- Provide a **range of research** on the topic – and not just the “good” data!
- **Critically analyse** a selected topic using a published body of knowledge (backed-up arguments)
- Provide an indication of what **further research** is necessary
- Identify areas of **controversy** in the literature



# How To Write A Scientific Literature Review?



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# Scientific Writing!



**...is writing about scientific topics aimed at specialists in a particular field**

Assume the reader is familiar with the research/topic area but not with the *specifics* of your review...

i.e. your lecturer

your Principal Investigator

peer-reviewers (journal articles, research papers, book chapters, grant proposals)

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*Use precision, clarity and objectivity!*



# Scientific Writing!



## 1. Be precise!

Ambiguities in writing cause confusion and may prevent a reader from grasping key concepts of your review...

- Use precise concrete language, no ambiguity  
eg '**correlated**' ≠ '**related**'
- Exclude similes/metaphors (and humour!)
- Be *quantitative* wherever relevant (stats, numbers etc.)

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# Scientific Writing!

## 2. Be clear!

Concepts in the sciences can often be complex; without clarity the reader may be confused or misled

- Simple language – no unnecessary “frills” (distractions)
- Pay attention to sentence structure, grammar

***Your reader will be interested based on the science only... make it easy for them to access!***



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# Scientific Writing!



## 3. Be objective!

Any claims that you make need to be based on facts, *not intuition or emotion*

- **Passive voice** – focus is on the literature!
- Avoid **assumptions** or sweeping statements
- Be aware of **research limitations** and refer to these in the review

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# How to Write a Scientific Literature Review?

Reviewing the literature requires four stages:

- 1. Problem formulation** - Which topic is being examined and why? What aspects will be included/excluded? Define your scope
- 2. Literature search** - Identifying relevant research
- 3. Critical analysis** – Criticise the experts; identify conflicting evidence, assumptions, errors and misconceptions
- 4. Evaluation** – which authors are most convincing and provide the most significant scientific contribution? Have I conducted a fair and objective literature review?





# 1. Problem Formation

Ask yourself questions like these:

- What useful reviews are **missing** or not up to date in my research area?
- What new review topic would be useful to scientists?
- Is there a **specific aspect of this topic** that my literature review might help to define?

*eg. critically comparing different methodological approaches, contrasting evidence, assessing therapeutic potential, etc.*

- What is the **scope** of my literature review? *Be specific*



# Literature Searching...



## 1. Online Research (basic) – Background Information

- Wikipedia (gasp!)
- Relevant “background” websites (eg. university websites, company websites, associations eg. American Heart Association)
- YouTube, TED Talks

## 2. General Literature Search – Literature Overview

- Google Scholar/Books
- PubMed

*...find other relevant literature reviews in the area to see what has been done/what is needed*

## 3. Specific Literature Search – The Detail

- Library databases *e.g Web of Science*
- “Advanced search” tool in Google Scholar/PubMed
- Identify key references for each topic of your review

**TIP: Use the Library!**

**Library staff are always there to help if you have questions on literature searching.**



## 3. Critical Analysis

In assessing each source, consideration should be given to:

- **Provenance** - Author's credentials? Are the author's arguments supported by evidence?
- **Objectivity** - Is the author's perspective fair? Is contrary data considered? Is information ignored to prove the author's point? (bias)
- **Persuasiveness** – Is the author's data convincing?
- **Value** - Does the work contribute in a significant way to an understanding of the field?

*...this involves **CRITICAL THINKING!***

A decorative graphic in the bottom right corner of the slide, consisting of several overlapping, semi-transparent geometric shapes in various colors: teal, green, orange, red, purple, and blue.

# What is critical thinking?

**Cottrell (2016):**

“The process of looking at ideas and information critically, taking nothing for granted, but questioning accuracy, motivation and inferences, and seeking new understanding, connections and insights.”



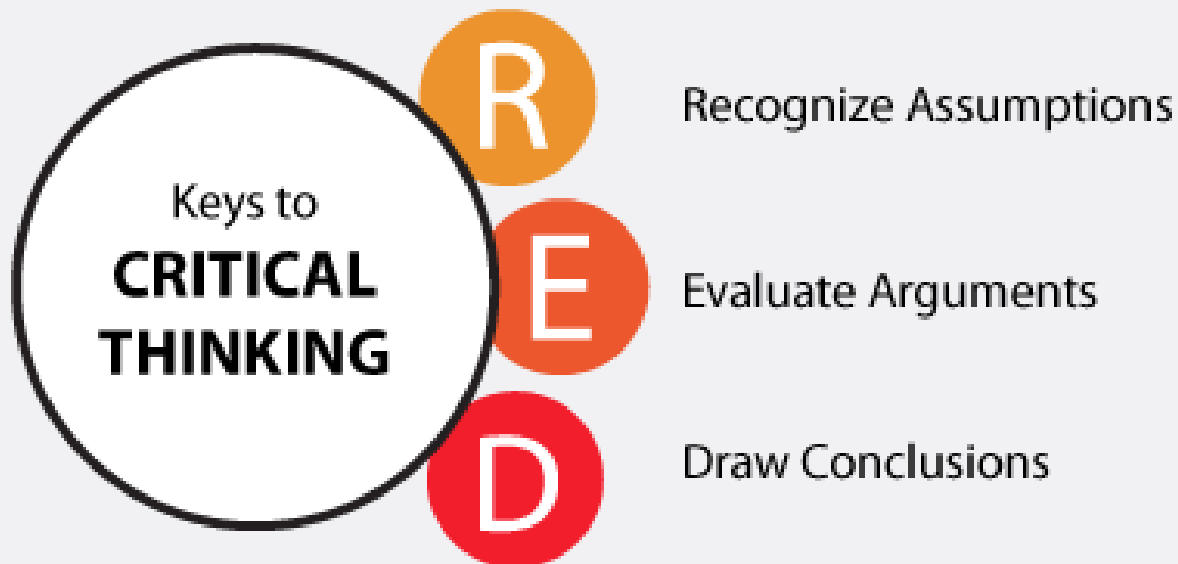
i.e. weighing up the evidence and arguments for or against something, and coming up with your own informed opinion.



# Ask questions!

- “Is that really true?”
- How do you know?
- Show me the evidence.
- Is that evidence reliable?”

“There is  
evidence on  
both sides”



Red Model based on the Watson-Glaser™ Critical Thinking Appraisal  
at [www.ThinkWatson.com](http://www.ThinkWatson.com)

# Critical Thinking...

Move from **Description** to **Analysis!**

## Description – reproducing information

- Summarising texts - accepting details, results etc.

## Analysis – deconstructing information in order to:

- *Challenge* assumptions; perspectives
- Show *limitations* in studies, exceptions to cases
- Highlight *under-examined* aspects of research



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# Key aspects of critical thinking

- Identify evidence to **back-up AND challenge** key points
- Detecting **inconsistencies and mistakes** in authors' reasoning
- Detecting **bias**, premature conclusions, lacking evidence
- Distinguishing between **fact and opinion**
- Evaluating **conflicting** opinions/research
- Suggesting new or different **solutions**
- Constructing **your own arguments and opinions**



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# What should I be asking?

- Why is the author choosing to use the evidence presented?
- Is there a hidden agenda? (*eg. financial gain*)
- Are the sources reliable and objective?
- Is there bias present?
- Have all of the points been cited?
- Is there information missing?
- Are there conflicting opinions/conclusions?

*And most importantly....*

- ***Do I agree with these opinions/conclusions?***



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# Critical Thinking...



Critical Thinking is the key to a good grade...

...don't be afraid to criticise the experts and show your understanding of the topic!

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**This is the most important aspect of a good literature review!**



## 4. Evaluation and Interpretation

- What **conclusions** can I make from the most convincing literature? What are my opinions/arguments?

***Also evaluate your own interpretations...***

- Have I made a well-informed decision? How good was my **information seeking**? Has my search been wide enough to ensure all relevant material is included? Has it been narrow enough to exclude irrelevant material?
- Have I **critically analysed** the literature I use?
- **Instead of just listing and summarizing research, do I assess them, discussing strengths and weaknesses?**
- Have I cited and discussed studies **contrary** to my perspective to form a well-balanced argument?



# Coherent Scientific Literature Reviews



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# Coherent Scientific Literature Reviews



Aim for:

- **Clear and cohesive** essay that integrates the key details of the literature and **communicates your point of view**
- Tackle **one key point** at a time
- Use **subheadings**, especially in long reviews
- Check the **flow** of your argument for coherence (logical order?)

*...this is all about **STRUCTURE!***

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# Scientific Literature Review:



## How to structure a scientific literature review?

- **Introduction:** An *overview* of the topic under consideration, along with the *objectives* of the literature review.
- **Main body:** Critical analysis, evaluation of topically relevant research/data; Break into **sub-headings**
- **Conclusion:** Summarise the **key points** from your review



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### Word count:

Introduction = 10%  
Main Body = 80-85%  
Conclusion = 5-10%



# Before you start writing...

## 1. Brainstorm/plan your review

Allow 10% of your word count for each Introduction and Conclusion

What are the key aspects of your review?

## 2. Decide on the number of “topics” you will address based on your remaining word count (80%)

Of the most interesting/relevant topics... how many can you address in the allowed word count? Prioritise!

## 3. Choose your topics

Scan the literature, make sure there is enough information out there for you to complete a coherent, critical summary of each chosen topic



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# 1. Introduction

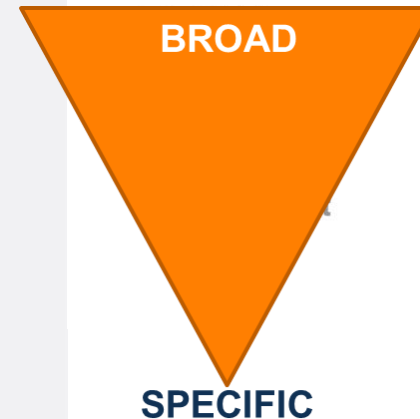
*It is usually easier to write this after the main body...*

Introduce your topic by highlighting the **core scientific facts** that are well backed up and widely accepted

Highlight the **importance** of the review – are you assessing potential clinical relevance? Gap in research area? New perspective?

What is the **core aim** of this review? To compare and contrast conflicting evidence? To identify under-examined aspects of the topic?

Tell the reader ***what you are going to talk about... list your topics in order!***



## 2. Writing the Main Body

- Group research topics according to common elements and back up main points with research
- Focus on **recent** data where possible – scientific fact changes/develops over time!
- Summarize individual studies or articles with as much or as little detail as is relevant – detail denotes significance!
- Tackle **one key point per paragraph** so as not to overwhelm the reader
- Use **sub-headings** to group your topics
- Use diagrams, figures, tables where appropriate





# Tackle 2-3 key points per section...



Sub-headings



<b>INTRO</b> 10% of word count	Go from the broad to the specific. Introduce the general topic, why it is an important area, then state what you will specifically do to investigate it further.		
<b>Section 1</b>	Sub-point 1	Sub-point 2	Sub-point 3
<b>Section 2</b>	Sub-point 1	Sub-point 2	Sub-point 3
<b>Section 3</b>	Sub-point 1	Sub-point 2	Sub-point 3
<b>CONCLUSION</b> 10% of word count	Go from the specific to the broad. State the conclusions you can draw from the points you've made in the essay, and connect this learning to the general topic. End by posing a question for future research in the field.		

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# ...one key point per paragraph!

## 1. Topic Sentence

- Start each topic with a strong “umbrella” sentence introducing your key point

## 2. Supporting Sentences

- Provide context, examples or data
- Each point backed up with a source/reference
- Use “linker” words to introduce similar points
- Opposing data should also be considered

## 3. Concluding Sentence

- Include summary sentences at end of paragraphs... why this information is relevant
- May link to following paragraph



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# 1. Writing the Main Body



[www.smart-words.org](http://www.smart-words.org)

## Agreement / Addition / Similarity

The transition words like **also**, **in addition**, **and**, **likewise**, **add information**, **reinforce ideas**, and **express agreement** with preceding material.

in the first place	again	moreover
not only ... but also	to	as well as
as a matter of fact	and	together with
in like manner	also	of course
in addition	then	likewise
coupled with	equally	comparatively
in the same fashion / way	identically	correspondingly
first, second, third	uniquely	similarly
in the light of	like	furthermore
not to mention	as	additionally
to say nothing of	too	
equally important		
by the same token		

## Opposition / Limitation / Contradiction

Transition phrases like **but**, **rather** and **or**, express that there is evidence to the **contrary** or point out **alternatives**, and thus introduce a change the line of reasoning (**contrast**).

although this may be true	but	although
in contrast	(and) still	instead
different from	unlike	whereas
of course ..., but	or	despite
on the other hand	(and) yet	conversely
on the contrary	while	otherwise
at the same time	albeit	however
in spite of	besides	rather
even so / though	as much as	nevertheless
be that as it may	even though	nonetheless
then again		regardless
above all		notwithstanding
in reality		
after all		

# Critical Phrases...



<http://www.phrasebank.manchester.ac.uk/>

## Introducing questions, problems and limitations: theory or argument

The main weakness with this theory is that ...  
The key problem with this explanation is that ...  
However, this theory does not fully explain why ...  
One criticism of much of the literature on X is that ...  
However, there is an inconsistency with this argument.  
A serious weakness with this argument, however, is that ...  
One question that needs to be asked, however, is whether ...  
Smith's argument relies too heavily on qualitative analysis of ...  
Smith's interpretation overlooks much of the historical research ...  
Many writers have challenged Smith's claim on the grounds that ...  
Smith's analysis does not take account of X, nor does he examine ...  
It seems that Jones' understanding of the X framework is questionable.  
The existing accounts fail to resolve the contradiction between X and Y.  
One of the limitations with this explanation is that it does not explain why...

## Identifying a study's weakness

(However,)

Smith fails to fully define what ...  
Jones fails to acknowledge the significance of ...  
the author overlooks the fact that X contributes to Y.  
what Smith fails to do is to draw a distinction between ...  
the paper would appear to be over-ambitious in its claims.  
another weakness is that we are given no explanation of how ...  
no attempt was made to quantify the association between X and Y.  
the main weakness of the study is the failure to address how ...  
the study fails to consider the differing categories of damage that ...  
the research does not take into account pre-existing ... such as ...  
the author offers no explanation for the distinction between X and Y.  
Smith makes no attempt to differentiate between different types of X.



## 2. Main Body: Figures/Tables

- Aim for one key figure/table per section; this can be to:
  - *illustrate a complex concept*
  - *summarise a large body of relevant data*
  - *describe the order of a process (flow diagrams)*
- Legend *below* image/figure and *above* table
- Always refer to figures/tables in text... direct the reader to them (*as seen in Figure 1; as summarised in Table 1*)
- Provide a detailed legend... each figure + legend should stand in its own right without the review text
- **Figures and tables provide a break for the reader and a chance to understand and reflect on key concepts!**



# Writing the Conclusion

- Maintain the focus established in the introduction
- Summarise major research contributions to the scientific field (most convincing data) and make your point of view clear
- Point out major flaws/gaps/inconsistencies in research
- Highlight potential future studies
- Provide closure so that the path of the argument ends with a conclusion of some kind

SPECIFIC

BROAD

**NOTE: A literature review in a thesis or dissertation usually leads to the research questions that will be addressed.... 4<sup>th</sup> Year students!**

# Additional Sections....

- Usually, a short **ABSTRACT** (approx. 200 words) is required before your literature text to summarise the topics, main findings and conclusions from your review
- *This tells the reader exactly what your review contains so that they can make an informed decision - if it is relevant or not - before reading the full text*
- **TABLE OF CONTENTS** – show the reader where to find the relevant information
- **ACKNOWLEDGEMENTS** – acknowledge any funding bodies/research groups that contributed to the review writing process
- **CONFLICT OF INTEREST** – you must declare if the *primary interest* of your review may be affected by any *secondary interests* (personal benefit)

# Revising & Editing

**Voice** – passive voice? Target audience?

**Cohesion** – sentence length/clarity?

**Criticality** – clear critical thinking?

**Referencing** – have I referenced where appropriate?

**Grammar** – Grammarly!

**Mechanical issues** – sentence length, spelling, punctuation

**Ask peers/family members** – get second/third/fourth opinion!

**Read out loud** - Claroread

**Give yourself a break** – Fresh eyes!

**YOU HAVE PUT IN SO MUCH TIME ALREADY....**

**....MAKE IT PERFECT!!!**



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# Referencing

It is essential to credit published papers for work mentioned in your manuscript...

- In-text
- Reference List/Bibliography – *what is the difference?*

“atherosclerosis has been claimed to be an independent risk factor for cardiovascular death (Detrano *et al.*, 2008)”.

Detrano R, Guerci AD, Carr JJ, Bild DE, Burke G, Folsom AR, Liu K, Shea S, Szklo M, Bluemke DA, O'Leary DH, Tracy R, Watson K, Wong ND, Kronmal RA. Coronary calcium as a predictor of coronary events in four racial or ethnic groups. *N Engl J Med* 2008. **358**:pp1336-1345.

Harvard referencing guide....

**CiteThemRight....**

Zotero referencing manager...

Mendeley/RefWorks – other options

**Do the library workshops!!**

*All available  
from DCU  
Library website*



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# Referencing

## Figures/Tables:

- In-text citation in the figure legend after description
- May need to ask for permission from the publisher – be careful! (*is the image copyrighted?*)
- If figure is adjusted: “image adapted from [source]”

**MAKE SURE YOU REFERENCE THE SOURCE MATERIAL (original research paper, where appropriate) and NOT A REVIEW OF THE RESEARCH**

Except when you are referencing another reviewer’s opinion/critique etc.

- Avoid plagiarism... use quotation marks for direct quotes + “in-text” citation
- Use “in-text” citation only to reference ideas/opinions/indirect quotes



# Example: Published Review...



Vascular Pharmacology 82 (2016) 30–40

Contents lists available at ScienceDirect  
**Vascular Pharmacology**  
journal homepage: www.elsevier.com/locate/yvph

Review

## Vascular calcification in type-2 diabetes and cardiovascular disease: Integrative roles for OPG, RANKL and TRAIL

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RANKL  
Vascular calcification  
TRAIL  
Type-2 diabetes mellitus

ABSTRACT

Vascular calcification (VC), a disorder that causes blood vessel hardening and dysfunction, is a significant risk factor for type-2 diabetes mellitus (T2DM), which invariably manifests associated cardiovascular complications. Although the clinical effects of VC have been well-documented, the precise cellular events underlying the manifestation and progression of VC are only now coming to light. Current research models indicate that VC likely involves signalling pathways traditionally associated with bone remodelling, such as the OPG/RANKL/TRAIL signalling system. In this respect, receptor activator of NF- $\kappa$ B ligand (RANKL) promotes VC whilst osteoprotegerin (OPG) acts as a RANKL decoy receptor to block this effect, events that contrast with the known functional influence of these proteins during bone metabolism. Moreover, evidence suggests that an alternative decoy ligand for OPG, TRAIL, may also be involved. In this review, we conduct a timely examination of these alternative perspectives. Our objectives are to (i) review the osteogenic and vascular calcificative roles of RANKL and TRAIL. Extensive *in vitro* findings highlighted; and (ii) to explore the potential role of TRAIL in the pathogenesis of VC. In this regard, a clear focus on the role of TRAIL in atherosclerosis will be made.

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Abbreviations: BMP, bone morphogenetic protein; VC, vascular calcification; OPG, osteoprotegerin; RANKL, receptor activator of nuclear factor- $\kappa$ B ligand; TRAIL, tumour necrosis factor-related apoptosis-inducing ligand.  
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http://dx.doi.org/10.1016/j.vph.2016.02.003  
1537-8891/© 2016 Elsevier Inc. All rights reserved.

Concise, informative title

Short abstract – 200 word summary

Table of Contents

E. Harper et al. / Vascular Pharmacology 82 (2016) 30–40 35

levels of OPG have been positively correlated with CAD [80] and peripheral vascular disease [81], whilst Omland and co-workers have highlighted its potential use as a predictor of heart failure and long term mortality in patients who suffer from acute coronary syndromes [82]. Most recently, Higgins and colleagues have demonstrated that both tissue and serum OPG are associated with calcification in human atherosclerosis [83]. OPG is a proposed inhibitor of VSMC proliferation [84], increased circulating OPG levels are associated with a tendency to tackle pro-inflammatory cytokines.

It has been shown that OPG levels increase shortly after initiation of diabetes [66], with a similar trend noted in clinical studies. Many studies have significantly correlated serum OPG elevation with worsening CV burden in T2DM, including CAC [85], carotid intima-media thickness [86], hypertension [87], coronary/peripheral arterial disease [88], metabolic syndrome and microvascular complications [89]. Elevated OPG has also been shown to invariably predict coronary artery VC progression in diabetics, and furthermore can be used to predict future CV events [90,91]. Finally, a 2012 study into advanced carotid atherosclerosis illustrated that a history of diabetes and CAD (among other diseases) could independently predict circulating plasma OPG levels [92]. Therefore, it is highly likely that serum OPG concentration may constitute an important and specific CVD biomarker in T2DM.

### 7.2. RANKL

Despite strong evidence supporting a role for OPG as a T2DM/CVD biomarker, clinical investigations focusing on RANKL have proven much more divisive, with varying clinical observations across the T2DM/CVD spectrum. It has been claimed for example that circulating RANKL levels are elevated in patients with either advanced carotid atherosclerosis [86], medial thickness [86]. More recently, RANKL has been positively correlated with atherosclerotic lesions [83]. With respect to T2DM, it has been reported that circulating RANKL levels are elevated in control subjects [86], whilst in T2DM patients, no change in plasma RANKL levels was observed. However, the authors identified an inverse relationship between RANKL and CAC triglycerides in post-menopausal women [93]. It has also been reported that RANKL expression is upregulated and localized to areas displaying medial arterial calcification in patients with Charcot neuroarthropathy [41], whilst soluble RANKL (sRANKL) has also been positively co-associated with well-known biomarkers of heart failure [94]. Interestingly, although it may not have intrinsic diagnostic value, Mohanadpour et al. have proposed the OPG:RANKL serum concentration ratio as a biomarker for CAD. In their ischemic coronary disease study cohort, they noted a significant correlation between OPG:RANKL and CAC [95]. Overall however, based on these recent clinical findings, a definitive role for RANKL as a serum biomarker for T2DM/CVD remains inconclusive.

### 7.3. TRAIL

There has been considerable clinical focus on the role of TRAIL in CVD. Secchiero and co-workers have found that TRAIL levels are decreased after acute myocardial infarction [96], and lower TRAIL levels are independently associated with increased risk of cardiac death in the year following patient discharge [97]. Consistent with the vasoprotective and anti-atherogenic properties previously postulated from *in vitro* and animal studies. Furthermore, due to elevated OPG and decreased TRAIL in acute MI patients, these researchers proposed that the ratio between OPG:TRAIL may have potential use as a biomarker, as this balance was significantly associated with CAD. In support of its efficacy as a biomarker, follow-up patients who developed heart failure had a significantly elevated OPG:TRAIL ratio than those who did not, indicating that this ratio may be used to predict heart failure in acute MI patients [96]. TRAIL levels have also inversely predicted all-cause mortality in patients with advanced heart failure [97]. In other research, Mori and co-workers reported that serum TRAIL levels were significantly lower in CAD patients, and were inversely associated with CAD severity independently of other coronary risk factors [98], while Volpato and colleagues found a significant inverse relationship between baseline serum TRAIL levels and all-cause CVD mortality [99]. Kawano and co-workers (2011) have also previously reported that serum TRAIL levels were significantly and inversely correlated with carotid intima-media thickness in a subset of T2DM patients with macrovascular diseases [100]. Notwithstanding these observations, inconsistencies between study findings are also evident from the literature. In this regard, O'Sullivan et al. found no change in TRAIL levels in T2DM subjects [78], whilst Galeone et al. detected high levels of TRAIL in calcified aortic valves, as well as elevated levels of circulating TRAIL in these CVD patients compared to control subjects [101]. The balance of clinical evidence however suggests that serum TRAIL levels may constitute an important predictor of CV burden in patients with T2DM and CVD.

### 8. VC – Interventional perspectives

The current perspective on VC management involves a variety of approaches. Thus far, however, there are no treatment options available for VC across the T2DM/CVD patient spectrum, most likely due to an insufficient understanding of the precise molecular and cellular mechanisms involved, in conjunction with a lack of human clinical studies. It is clear that the dynamic pathways involving OPG, RANKL and TRAIL represent potential therapeutic targets for interference of the calcification process. To date, however, progress in exploring these therapeutic options (which could play a key role in the development of an effective treatment for VC) has been limited. Nonetheless, the anti-calcific effects of OPG/TRAIL, as well as the pro-calcific effects of RANKL, have been considered by some authors in the context of generating targets for VC intervention, and are discussed below.

#### 8.1. Recombinant OPG therapy

Unsurprisingly, in view of its mechanism of action, OPG administration has been suggested as one potential treatment option for VC [102]. OPG functions to prevent osteoclastogenesis and resorption in bone, whilst also having a paradoxical function in preventing osteochondroblastic calcification within the vasculature, thus resulting in a context-specific dual protective function. In support of this, numerous murine studies have illustrated that OPG deficiency tends to increase the extent of VC and cardiovascular complications, and promisingly, a recombinant OPG fusion protein (Fc-OPG) has been shown to inhibit VC in an animal study [84]. In this latter study, *Idhr<sup>-/-</sup>* mice were fed an atherogenic diet alongside Fc-OPG administration; calcifi-

Sub-headings

Good paragraph length to clearly analyse key topics

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# Example: Published Review...



fully delineated, and alongside these reports, additional studies point to the involvement of three specific glycoproteins; OPG, RANKL and tumour necrosis factor-related apoptosis-inducing ligand (TRAIL). The following sections will examine the evidence for involvement of these glycoproteins within the VC process, including proposed cellular mechanisms arising from *in vitro* and animal study models.

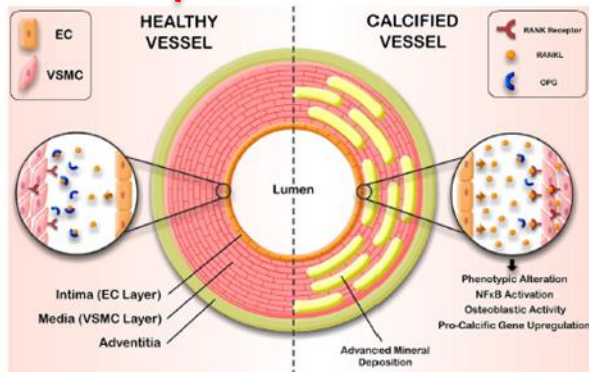
#### 4. VC – OPG, RANKL and TRAIL

There are numerous molecular components to the VC signalling cascade, described in detail by Sage and colleagues [40], many of which are related to bone morphogenesis. There is growing evidence that the OPG/RANKL/RANK signalling axis is central to VC manifestation [37]. RANKL actively promotes the calcification process in vascular cells by inducing osteoblastic activity [27]. RANKL, when secreted by endothelial cells (ECs), can bind to the RANK receptor to promote pathological differentiation of healthy VSMCs into calcified VSMCs with an osteoblastic phenotype [27,41,42]. In this respect, RANKL is upregulated in calcified VSMCs [42] and has been shown to exert its pro-calcification actions through activation of the alternative NF- $\kappa$ B pathway [27]. Thus, when serum entrapment process of medial arterial wall acts as a soluble decoy for RANKL, an event anti-calcific effect RANKL and OPG app calcification to those ing bone remodel

**Informative/relevant image and figure legend**

a vasoprotective role for TRAIL, possibly through pleiotropic effects on vascular gene expression and/or an ability to mediate RANKL signalling; contrastingly, however, some competing theories point to a potential role for TRAIL as an inducer of calcification. As its name suggests, TRAIL is an apoptosis-inducing protein of the TNF ligand superfamily, and is thus far known to be expressed by immune and vascular cells [45,46]. TRAIL is a type-II transmembrane protein with the ability to bind five different receptors found on numerous cell types, as well as a C-terminal domain that can also be cleaved from the cell surface to release a soluble form. Two TRAIL receptors (DR4 and DR5) have a cytoplasmic death domain, whilst two decoy receptors (DcR1 and DcR2) lack a functional death domain; thus TRAIL-induced apoptosis via DR4 and DR5 is antagonized by the competitive inhibitory effect of DcR1 and DcR2. OPG acts as an additional decoy receptor for TRAIL (and vice-versa); therefore, OPG has a second protective function (i.e. in addition to its ability to block RANKL-induced calcification) by virtue of its ability to block TRAIL-dependent apoptotic signalling [47]. TRAIL and its receptors have been identified in vascular endothelial and smooth muscle cells, as well as both healthy and injured arterial wall [38], however its precise roles within the vasculature are as yet unclear. TRAIL, by its ability to induce apoptosis, may play a role in the pathophysiology of atherosclerosis, and its potential role in the pathophysiology of VC is poorly defined. In this regard, an emerging hypothesis within the VC field has proposed the vascular system, a fact which may be pertinent in explaining the apparent contradictions in TRAIL function. Overall, there is evidence to suggest that TRAIL has substantive yet diverse functional roles within the vasculature, both dependent on and independent of OPG and RANKL.

Interestingly, a third regulatory protein, TRAIL, has been shown to interact with OPG and RANKL during modulation of the VC process [44], although its precise functions in this context are poorly defined. In this regard, an emerging hypothesis within the VC field has proposed



**Fig. 2.** Vascular calcification. In the vasculature, the EC monolayer releases baseline levels of soluble RANKL. OPG, predominantly secreted by VSMCs, binds and neutralizes RANKL in the intraluminal space, preventing RANKL interaction with membrane-bound RANKL on the VSMC surface. Thus, phenotypic alteration of the VSMC layer is prevented, resulting in a healthy non-calcified vessel (left). Alternatively, when soluble RANKL levels are high, VSMCs cannot secrete sufficient OPG to neutralize the excess. RANKL interacts with RANKL on the VSMC surface, forming a RANKL/RANKL complex that initiates VSMC trans-differentiation. NF- $\kappa$ B activation, osteoblastic/chondroblastic activity and pro-calcific gene upregulation ensues, finally resulting in advanced mineral deposition and calcification within the VSMC medial layer (right). EC, endothelial cell; VSMC, vascular smooth muscle cell; RANKL, receptor activator of nuclear factor kappa-B; RANKL, receptor activator of nuclear factor kappa-B ligand; OPG, osteoprotegerin; NF- $\kappa$ B, nuclear factor kappa-B.

bone degradation and ultimately resulting in low bone mass and fragility [106]. Denosumab, a human monoclonal antibody for RANKL, is one of the latest approved treatment options for osteoporosis [102,107], although its effects on VC have not yet been fully assessed. Mimicking the natural actions of OPG, Denosumab binds and neutralizes RANKL (but not TRAIL), attenuating its osteoclastic effects and allowing osteoblastic build-up of bone to ensue [108]. As RANKL promotes osteochondroblastic activity in VSMCs, anti-RANKL therapy could theoretically function to reduce the extent of calcification in the vasculature. In support of this theory, it has been demonstrated that Denosumab reduced aortic calcium levels by half in a murine model of osteoporosis [109], but contrastingly human study completed to date has not shown any effect on aortic calcification progression [110]. It is possible that this disparity is due to differences in calcification measurement, as Samelson and colleagues [110] used a quantitative method (lateral spine X-rays) whereas the study by Denosumab and co-workers. Furthermore, this study was initially completed to assess the effect of Denosumab on bone mineral density in osteoporotic postmenopausal women

(2363 of 7808 patients). The therapeutic potential of anti-RANKL therapy for the treatment of VC therefore awaits further clinical investigation.

#### 8.3. TRAIL administration

Although its potential therapeutic use in cardiovascular protection has been suggested [99], there have been no human clinical investigations conducted to date that address the potential of TRAIL for the treatment of VC. As noted however, recombinant TRAIL administration to ApoE<sup>-/-</sup> diabetic mice has been shown to significantly reduce atherosclerosis progression [67], whilst TRAIL delivery protects against diabetic vascular in-

**Clear summary table and table legend**

**Table 1**  
Potential therapies for the inhibition/reversal of VC. Key: CAC, coronary artery calcification; GLP-1RA, glucagon-like peptide-1 receptor agonist; OPG, osteoprotegerin; RANKL, receptor activator of nuclear factor kappa-beta ligand; TZDM, type 2 diabetes mellitus; TNF, tumour necrosis factor-related apoptosis-inducing ligand; VC, vascular calcification; VSMC, vascular smooth muscle cell. \*Denosumab can be classified as both an OPG/RANKL/TRAIL inhibitor and an osteoporosis therapy.

Therapy	Mode of action	Results to date	References
<b>OPG/RANKL/TRAIL-related therapies</b>			
Denosumab*	Neutralizes RANKL; prevents phenotypic transformation of vascular cells.	Decreased aortic VC in a murine study; no effect on calcification in a human sub-analysis of a larger trial.	[109][110]
Recombinant OPG Therapy	Neutralizes RANKL; prevents phenotypic transformation of vascular cells.	Inhibited VC in a murine study.	[84]
TRAIL Administration	Unclear	Reduced atherosclerosis progression in a murine model; protected against diabetic vascular injury in a rat model.	[67][111]
<b>Osteoporosis therapies</b>			
Bisphosphonates	Prevents calcium and phosphate release from bone; inhibits crystal nucleation and propagation.	Suppressed calcification in a rat model; conflicting data in human studies.	[113][114][115]
Teriparatide	Upregulates circulating concentrations of osteonectin, a calcification inhibitor.	Decreased valve calcification in murine studies.	[116]
<b>Cardiovascular disease therapies</b>			
Statins	Prevent dyslipidemia and inflammation, risk factors for VC.	Protective effects on VC in a rat model; conflicting data in human studies.	[117][118][121][122]
Endothelin receptor agonists	Reduces hypertension, a risk factor for VC.	Significantly reduced VC in a rat model.	[126]
Interleukin-1 $\beta$	Reduces inflammation, a risk factor for VC.	Attenuated calcification in a murine model.	[127]
<b>TZDM therapies</b>			
Exenatide (GLP-1RA)	Enhances glucose-dependent insulin secretion to reduce TZDM symptoms.	Attenuated VSMC calcification <i>in vitro</i> ; no <i>in vivo</i> studies completed to date.	[124]
Liraglutide (GLP-1RA)	Enhances glucose-dependent insulin secretion to reduce TZDM symptoms.	No decrease in calcification noted in one prospective observational study to date.	[128]
<b>Chronic kidney disease therapies</b>			
Phosphate binders	Decreases circulating concentrations of phosphate.	Conflicting data, but favouring reduced progression of calcification with non-calcium based phosphate binders.	[129]
Calcimimetics	Lower circulating calcium levels.	Reduced mortality in uremic rats; reduced VC in humans in combination with low-dose vitamin D.	[130][131][132]
Vitamin D receptor agonists	Mechanism not fully understood, but shown to increase osteopontin expression.	Significantly reduced aortic calcification in a murine model.	[133]
Vitamin K	Upregulates production of MGP, which binds calcium ions.	Prevented arterial calcification in a rat model; slowed the progression of CAC in healthy older adults with pre-existing CAC in one human study.	[134][135]
Sodium thiosulfate	Chelates calcium, reduces inflammation.	Prevented calcification in a uremic rat model; uncertain if suitable for VC treatment in humans. Recognized treatment for calciphylaxis.	[136][137][138]

# Example: Published Review...



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of other emerging concepts for manipulating VC, some of which are related to current treatments for osteoporosis, CVD, and chronic kidney disease (CKD) [102]. Firstly, like Denosumab, bisphosphonates (pyrophosphate analogs) are a successful osteoporosis treatment that have been considered as a potential VC therapy option due to their inhibitory effect on hydroxyapatite crystal formation [112]. Although animal studies have shown promise [113], human studies involving bisphosphonates and calcification have revealed mixed results [114,115]. Additionally, teriparatide, a shortened recombinant human parathyroid hormone also employed for osteoporosis treatment, has been shown to reduce VC in *ldlr<sup>-/-</sup>* mice [116], although to the best of our knowledge, no teriparatide studies in humans have emerged in the literature to date. Due to overlap in the molecular mechanisms involved in osteogenesis and calcification, it is likely that further investigation into these currently existing osteoporosis treatments may aid in the development of an efficacious treatment for VC.

Statins, which have been routinely employed to lower blood cholesterol and prevent vascular complications associated with CVD and T2DM, have also been considered as a potential treatment option for VC, in view of their inherent pleiotropic properties [102]. In this respect, studies thus far have demonstrated conflicting results. Statin-treated patients were shown to have reduced aortic stenosis in an early investigation [117], and more recent studies have illustrated a protective influence of statins on VC in rats [118]. Additionally, statins have been shown to reduce levels of pro-calcific serum RANKL [119] and to increase anti-calcific serum OPG [120]. Elsewhere, it has been claimed that statins do not affect aortic stenosis with calcification [121], while a recent study has suggested that statins actually promote coronary atheroma calcification [122]. Further investigation is clearly warranted in order to resolve this ongoing debate and determine if the pleiotropic effects of statins can successfully reduce VC.

Additionally, Glucagon-Like Peptide-1 Receptor Agonists (GLP-1RAs), a new class of injectable glucose-lowering drugs which function through the incretin system in the gut, are currently employed in T2DM treatment and exhibit simultaneous cardioprotective effects [123]. Recently, Zhan and colleagues examined the effect of exenatide, a GLP-1RA, on VSMC calcification *in vitro*. Their results illustrated an attenuation of osteoblastic differentiation and calcification of VSMCs in both a time- and dose-dependent manner, alongside a decrease in the expression of RANKL. It was concluded that exenatide can inhibit

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## 10. Conclusions

There is currently a strong need to fully define the molecular mechanisms underpinning the development and progression of VC, a major risk factor for T2DM and CVD, in order to develop appropriate therapeutic approaches. Research emerging through *in vitro*, *in vivo*, and clinical studies now indicates that OPG, RANKL, and TRAIL, regulatory glycoproteins typically associated with bone remodelling, are of fundamental relevance to the process of VC. It is likely that some or all of these

proteins may prove diagnostically useful as circulating biomarkers that may be employed to stratify patients with respect to VC severity – from newly diagnosed T2DM sufferers to individuals with more well established T2DM and pre-existing CVD complications. In addition, the potential of these glycoproteins as molecular targets for treating VC, alongside currently existing therapies for osteoporosis, CVD and CKD, is attracting considerable attention, as evidenced within the scientific literature.

## Conflict of interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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Relevant acknowledgements

Lengthy reference list

Concluding with key points and future work



# QUESTIONS

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