

Introduction

Wound Healing in Type 2 Diabetes

- Insulin resistance in T2D is associated with several changes which contribute to delayed wound healing.
- Non-healing foot ulcers develop in 10-25% of diabetics during their lifetime and overall 5% per year require amputations.

Ganglioside GM3

- Ganglioside GM3 is a glycolipid (i.e. a fat combined with a sugar), known to regulate skin cell growth and migration.
- The Paller laboratory has found that GM3 levels are increased in the skin in T2D.
- It is hypothesized that the increased levels of GM3 in the skin is associated with poor wound healing and insulin resistance.
- It was found that an increase in GM3 correlates with slow migration of and that a reduction correlates with accelerated migration of keratinocytes in monolayer keratinocyte cultures – even in the face of high glucose.

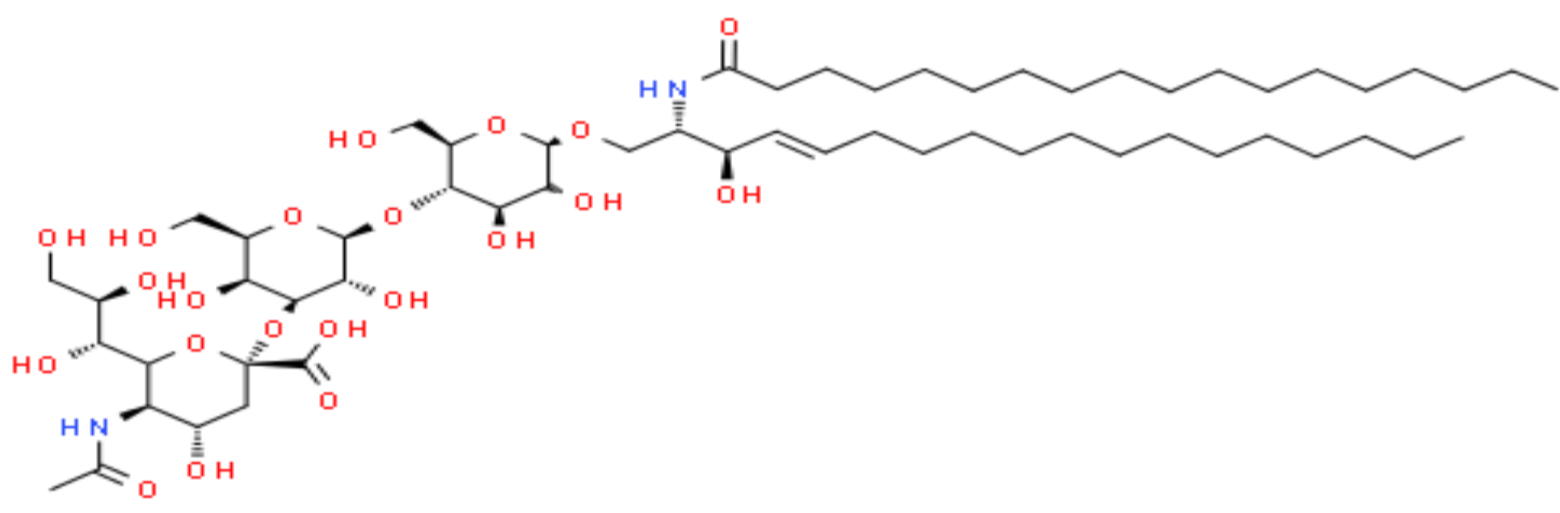


Figure 1 – Ganglioside GM3

CD 31

- CD31 is a specific receptor that is only present on the surface of the cells that make up the inner lining of blood vessels (endothelial cells).
- CD31 can be therefore used as a marker of blood vessels
- The density of the CD31 staining in the wound tissue is a measure of the amount of blood vessels that are present in the tissue.
- Angiogenesis was measured by **CD31 Density in Wound Tissue at day 7 post wounding.**

Aims

1. Investigate if changing levels of GM3 in the skin had an effect on angiogenesis in the wound tissue. [Experiment 1]
- By knocking out the gene that produces the enzyme that makes GM3 we can see the effect having no GM3 would have [GM3SKO].
1. Investigate if modulation of levels of GM3 using GZ (novel treatment) had a significant effect on angiogenesis in the wound tissue. [Experiments 2 & 3]

Methodology

- The skin of mice fed with a high fat diet can exhibit a diabetic phenotype. That is to say that the skin of the mice should behave in a similar way to the skin of a diabetic human.
- The sole of a mouse's feet best resembles human skin.

1) Generation of Samples:

- The mouse work to produce and fixate the samples, as well as its processing into slides was carried out by other members of the Paller lab.

2) Imaging:

- The samples were imaged using a Bright-field microscope, on the TissueQuest software platform.

3) Analyzing Images:

- The images on the slides were exported to be analyzed using HistoQuest software platform.
- The total wound area was calculated as the wound tissue has a different appearance under the microscope to the normal skin tissue.
- NB Granulation tissue was not used as it requires eosin counterstain and CD31 was already the counterstain.
- The area of CD31 staining in the wound tissue was measured.

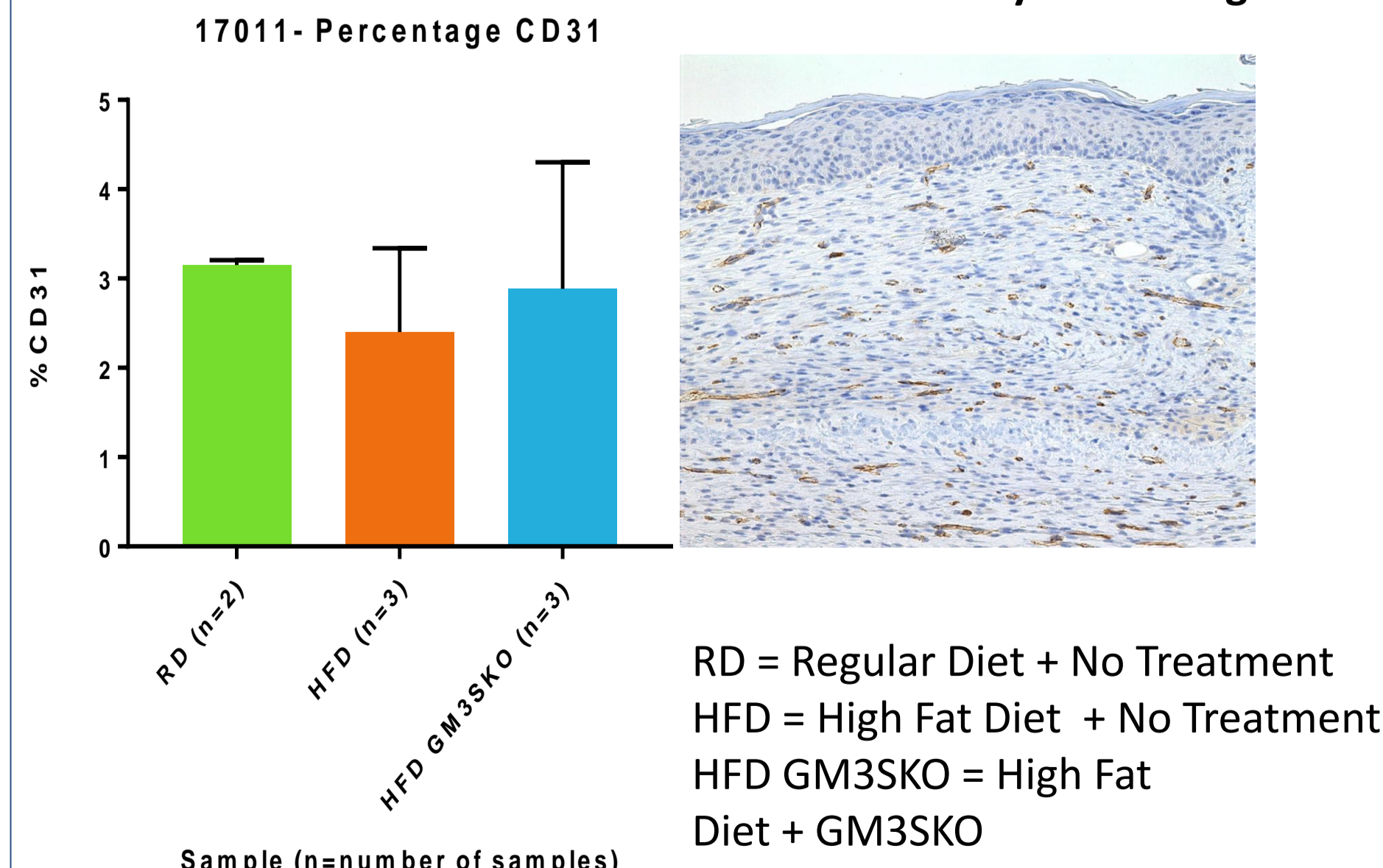
4) Calculation of CD31:

- The density of CD31 staining was calculated by working out the percentage the area of CD31 staining was of the total wound tissue area.

Results

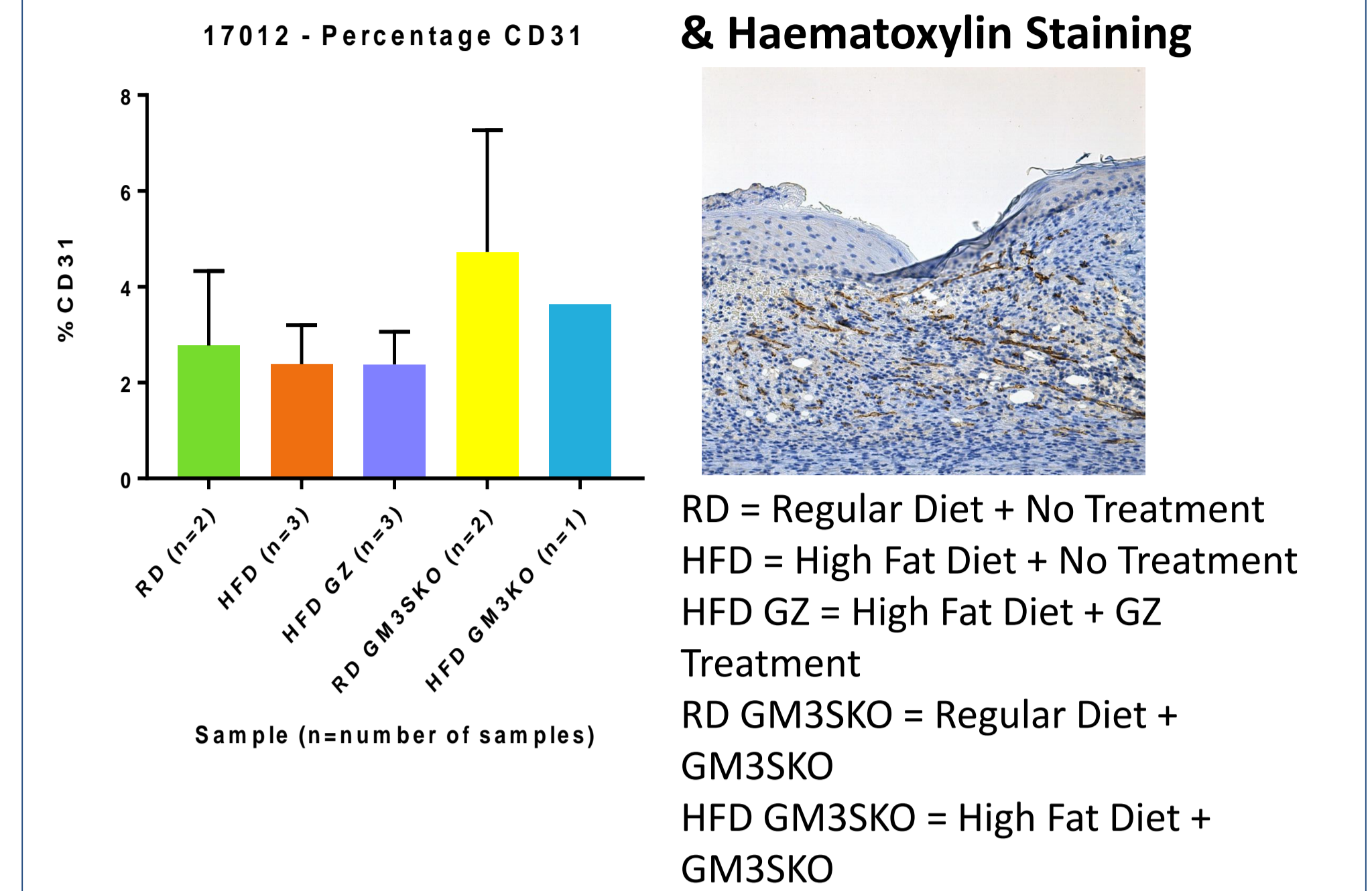
CD 31 Density - Angiogenesis

Experiment 1:



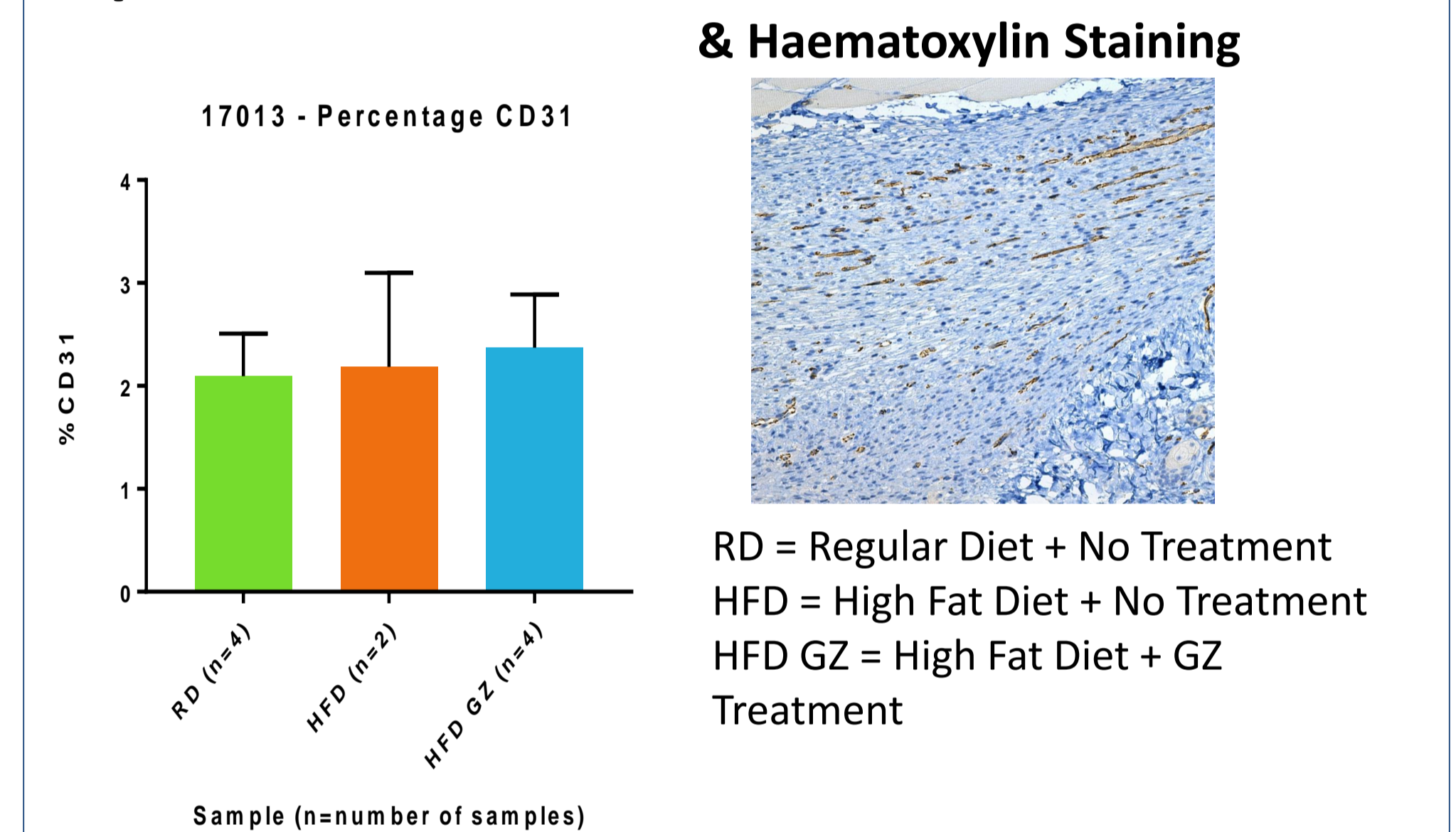
- HFD seems to have a lower CD31 density than the other two groups.
- There is no statistically significant difference between the three groups (high p value and large error bars).

Experiment 1:



- RD GM3SKO vs RD shows a possible trend.
- HFD GM3SKO vs HFD show a possible trend.
- There is no statistically significant difference between the three groups (high p value and large error bars).

Experiment 1:



- There is no statistically significant difference between the three groups (high p value and large error bars).

Discussion

- The trend of the CD31 density in the GM3 Synthase Knockout RD versus RD and in the GM3 Synthase Knockout HFD versus HFD suggests that the modulation of this pathway might have a proangiogenic effect on the wound healing process.
- There is also a trend in Experiment 1 and 2 to suggest there might be a difference in CD31 density between RD and HFD. In T2D, slower formation of new blood vessels through angiogenesis in the wound tissue would be expected.
- GZ does not appear to improve angiogenesis in the HFD versus no treatment control in Experiments 2 & 3.
- For any of the trends to be validated more samples are required.

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