Reduction of Skin and Food Autofluorescence in Different Mouse Strains through Diet Changes

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Introduction

Autofluorescence, i.e. unwanted light emission from unlabeled tissues and/or ingested food, is one of the greatest weaknesses to the value of in vivo molecular imaging because it increases the difficulty of detecting weak signals. Oils, pigments, and proteins endogenous to mice, such as collagen, elastin, and beta carotene, contribute to whole body autofluorescence. However, the largest component of the unwanted autofluorescent signal is usually chlorophyll, which is found in most plant based murine chow diets. By comparing three commonly used strains of mice, as well as purified and chow diets, we were able to show that elimination of chlorophyll from the diet not only decreases the chlorophyll fluorescence component in the abdominal region, but also over the entire surface of the mouse. Chlorophyll free food, regardless of whether it is a chow or purified diet, significantly reduces autofluorescence by preventing the incorporation of chlorophyll into the rest of the body. The mice were imaged at several time points in the CRi Maestro Multispectral Imaging System using six different filter sets – we focus on the yellow filter set in the present study because of the prevalent use of Cy5.5 as a fluorescent probe.

Summary and Conclusions

- All diets examined significantly reduced chlorophyll fluorescence over the whole body of the mouse, in the abdominal region as well as all other areas. Reduction of whole body autofluorescence enables detection of weak fluorescent signals, effectively increasing the sensitivity of the optical detection system.
- The corn and soy chow (Harlan Teklad 2918) and the corn only chow (Harlan Teklad 2916) reduced autofluorescence as well as the purified diets (Research Diets D10001 and D10012G). With the elimination of chlorophyll fluorescence, nutritional and financial concerns become the deciding factors in diet choice.
- Seven days is sufficient time to reduce skin autofluorescence and reduce gut localized chlorophyll fluorescence below detectable levels.

Materials and Methods

Mice Strains

C57BL/6
- black furred
- most commonly used for transgenic models of disease

CD-1
- white furred
- most commonly used outbred strain

HSD nu/nu
- no fur
- most commonly used in oncology research for tumor study

Diets

Standard Chow: Lab Diets (Purina) 5053

Purified: Harlan Teklad TD97184
Research Diets D10001
Research Diets D10012G
(for breeders)

Alternative Chows:
Harlan Teklad 2916 (corn)
Harlan Teklad 2918 (corn & soybean)

Terminology

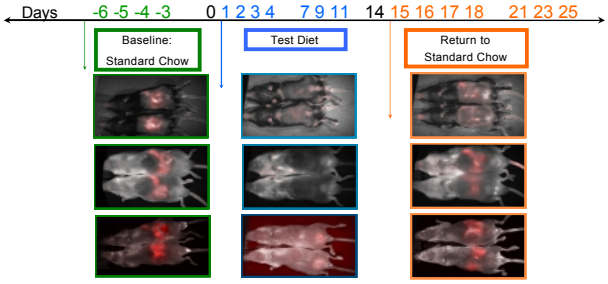
Skin Autofluorescence: Autofluorescence due to endogenous murine chromophores.

Chlorophyll Fluorescence: Fluorescent spectrum due to chlorophyll in standard murine chow.

Imaging Details

Yellow filter set – NIR region
-Recommended for commonly used Cy5.5 dye
-Excitation Filter: 575 - 605nm
-Emission Filter: 645nm longpass
-Exposure time optimized for each strain during baseline imaging.

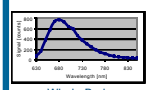
Experimental Design



In the time course study (data shown in the panel below) mice from each strain were imaged before (Baseline), during (Test Diet), and after (Return to Standard Chow) being fed Harlan Teklad's chlorophyll free purified diet, TD97184. The Test Diet and Return to Standard Chow phases each lasted 11 days. After having established an optimal imaging time course from the time course study, we compared whole body autofluorescence at days 3, 7, and 11 in mice fed four other chlorophyll free diets - two purified and two chow (data shown in panel at right). Images were captured with a CRi Maestro™ Multispectral Imaging System. The CRi Maestro™ software allowed for spectral unmixing of multiplexed fluorescent spectra.

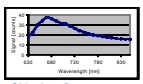
Baseline images provided the whole body autofluorescence spectrum. The skin autofluorescence spectrum was isolated from day 11 images, when there was no chlorophyll presumed present. This spectrum was used to spectrally unmix the skin autofluorescence and chlorophyll fluorescence from the whole body autofluorescence spectrum. Determination of unique spectra for each component proved straightforward once the experimental objectives had been clearly defined.

Measurement of dynamic spectral intensities was accomplished using small, circular ROIs of arbitrary size. The chlorophyll component was measured in two regions of the mouse body: gut (placed over the highest signal in abdomen) and skin (placed in the shaved region just above the stomach). Skin autofluorescence was measured in regions away from known food accumulation sites.



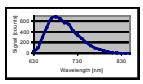
Whole Body Autofluorescence

Measured from the abdominal region (shaved for the furred strains) in baseline images. Averaged across all baseline images for each strain.



Skin Autofluorescence

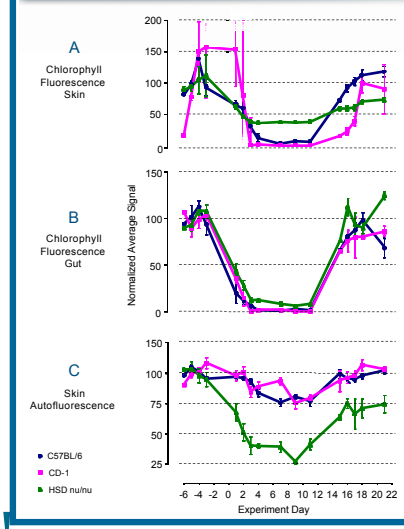
Measured from the abdominal region (shaved for the furred strains) in images acquired after the mice had been fed chlorophyll free food for 11 days. Averaged across all images for each strain.



Chlorophyll Fluorescence

Unmixed using the CRi Maestro™ Manual Compute Spectra Tool, which deconvolves a known auto-fluorescent skin signal from a multiplexed signal.

Strain Dependent Autofluorescence Decrease

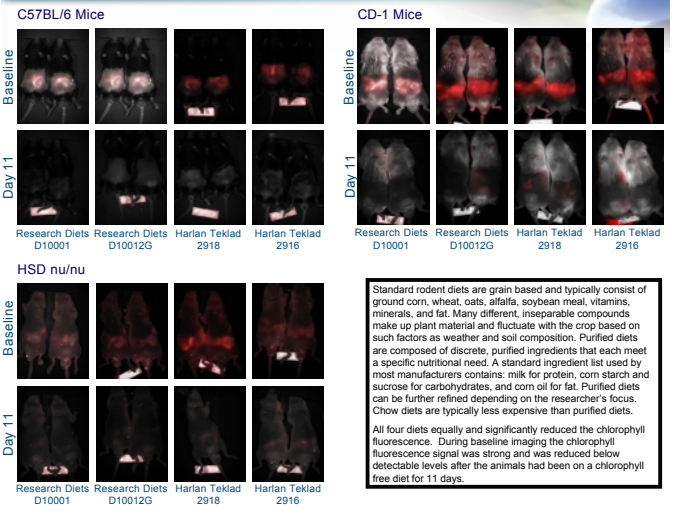


The trend in figure A matches that of the decrease of chlorophyll fluorescence measured in the gut, figure B. Average signal drops to nearly zero from baseline values after only 4 days on chlorophyll free food.

Chlorophyll fluorescence measured away from known food accumulation sites decreases as mice are fed a chlorophyll free diet.

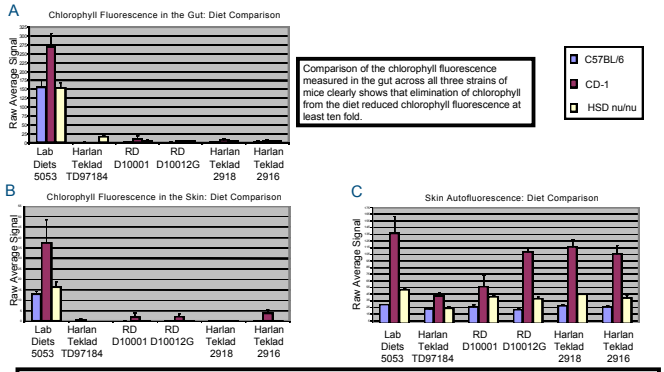
Skin autofluorescence remains nearly constant in the C57BL/6 and CD-1 mice, regardless of which diet they are fed (figure C). The skin autofluorescence of the HSD nu/nu fluctuates with the diet changes.

Comparison of Rodent Diets



Standard rodent diets are grain based and typically consist of ground corn, wheat, oats, alfalfa, soybean meal, vitamins, minerals, and fat. Many different, inseparable compounds make up plant material and fluctuate with the crop based on such factors as weather and soil composition. Purified diets are composed of discrete, purified ingredients that each meet a specific nutritional need. A standard ingredient list used by most manufacturers contains: milk for protein, corn starch and sucrose for carbohydrates, and corn oil for fat. Purified diets can be further refined depending on the researcher's focus. Chow diets are typically less expensive than purified diets.

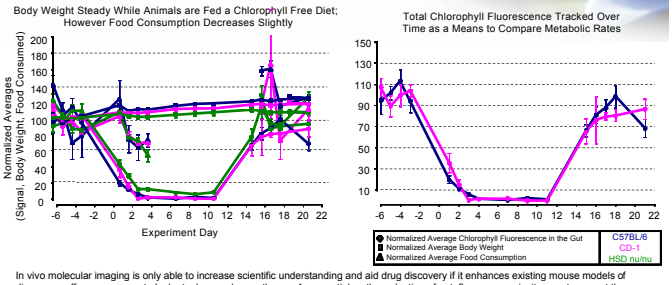
All four diets equally and significantly reduced the chlorophyll fluorescence. During baseline imaging the chlorophyll fluorescence signal was strong and was reduced below detectable levels after the animals had been on a chlorophyll free diet for 11 days.



Comparison of the chlorophyll fluorescence measured in the gut across all three strains of mice clearly shows that elimination of chlorophyll from the diet reduced chlorophyll fluorescence at least ten fold.

Elimination of chlorophyll from the diet also significantly reduced the chlorophyll fluorescence emitted from the skin, figure B. The inherent skin autofluorescence remains relatively constant across all the diets in the C57BL/6 and HSD nu/nu mice. The CD-1 mice consistently appear to have larger skin autofluorescence signals than the other strains. CD-1 autofluorescence decreases while on two of the purified chlorophyll free diets, but not on the third nor either on the chlorophyll free chow diets, figure C. The larger CD-1 skin autofluorescence signal is likely due to a higher level of reflective fur (data not shown).

Weight and Metabolism



In vivo molecular imaging is only able to increase scientific understanding and aid drug discovery if it enhances existing mouse models of disease or offers a new way to look at a known drug pathway. As essential as the reduction of autofluorescence is, it cannot come at the price of altering the normal metabolic balance of the mouse under study. While the average fluorescence signal decreases when the mice are fed chlorophyll free food, they continue to steadily gain weight, thus maintaining the natural growth pattern. However, it does appear that the taste of the food may be less appealing or require adjustment because food consumption decreases immediately following the switch to chlorophyll free food, and then is initially increased above baseline levels (food consumption was only measured during the first four days on chlorophyll free food and for the first four days after the standard chow diet resumed). It will be important in future studies to compare food consumption between multiple chlorophyll free diets to see if a chlorophyll free chow diet is consumed a rate closer to that of the standard diet. At present it is not yet possible to sufficiently quantify metabolic rate beyond the general statement that it is quite fast in C57BL/6 and CD-1 mice because the total chlorophyll fluorescence signal in the gut, indicative of the presence of chlorophyll containing food, has been reduced below detectable levels by day four.

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