Supplementary Materials

# Effect of dietary docosahexaenoic acid on rhodopsin content and packing in photoreceptor cell membranes

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**Table S1. ANOVA and Tukey's post-hoc analysis of DHA quantification data in Table 1**

| | *p*-value | | | | | | |
|---|---|---|---|---|---|---|---|
| | ANOVA | Control vs DHA-adq | Control vs DHA-def | Control vs DHA-rep | DHA-adq vs DHA-def | DHA-adq vs DHA-rep | DHA-def vs DHA-rep |
| DHA (µg/mg retina) | <0.0001 | 0.0006 | < 0.0001 | 0.0666 | < 0.0001 | 0.0702 | <0.0001 |
| DHA (µg/mg protein) | < 0.0001 | 0.0019 | <0.0001 | 0.3856 | <0.0001 | 0.0333 | <0.0001 |

**Table S2. ANOVA and Tukey's post-hoc analysis of scotopic a-wave data in Fig. 1D**

| Flash intensity (log cd·s/m$^2$) | *p*-value | | | | | | |
|---|---|---|---|---|---|---|---|
| | ANOVA | Control vs DHA-adq | Control vs DHA-def | Control vs DHA-rep | DHA-adq vs DHA-def | DHA-adq vs DHA-rep | DHA-def vs DHA-rep |
| -0.6 | 0.0082 | 0.8264 | 0.0083 | 0.3105 | 0.0341 | 0.7533 | 0.2225 |
| 0 | 0.0003 | 0.7035 | 0.0004 | 0.1568 | 0.0022 | 0.6256 | 0.0288 |
| 0.6 | < 0.0001 | 0.8581 | < 0.0001 | 0.1777 | 0.0002 | 0.4881 | 0.0031 |
| 1.4 | 0.0050 | 0.8258 | 0.0343 | 0.8711 | 0.1383 | 0.3477 | 0.0042 |
| 2.1 | 0.0300 | 0.8905 | 0.1811 | 0.8029 | 0.4617 | 0.3483 | 0.0216 |

**Table S3. ANOVA and Tukey's post-hoc analysis of scotopic b-wave data in Fig. 1D**

| Flash intensity (log cd·s/m$^2$) | *p*-value | | | | | | |
|---|---|---|---|---|---|---|---|
| | ANOVA | Control vs DHA-adq | Control vs DHA-def | Control vs DHA-rep | DHA-adq vs DHA-def | DHA-adq vs DHA-rep | DHA-def vs DHA-rep |
| -3.6 | 0.0201 | 0.4504 | 0.0269 | 0.9711 | 0.3525 | 0.6677 | 0.0461 |
| -3.0 | 0.0347 | 0.6466 | 0.0519 | 0.9950 | 0.3462 | 0.7520 | 0.0602 |
| -2.4 | 0.0140 | 0.7637 | 0.0818 | 0.8722 | 0.3617 | 0.2914 | 0.0113 |
| -1.8 | 0.0002 | 0.7830 | 0.0024 | 0.7980 | 0.0110 | 0.2404 | 0.0002 |
| -1.2 | < 0.0001 | 0.7446 | < 0.0001 | > 0.9999 | 0.0002 | 0.6839 | < 0.0001 |
| -0.6 | < 0.0001 | 0.9116 | < 0.0001 | 0.9769 | < 0.0001 | 0.9930 | < 0.0001 |
| 0 | < 0.0001 | 0.8265 | < 0.0001 | 0.8847 | < 0.0001 | 0.9990 | < 0.0001 |
| 0.6 | < 0.0001 | 0.8244 | < 0.0001 | 0.6822 | 0.0001 | 0.9924 | 0.0002 |
| 1.4 | 0.0001 | 0.6810 | 0.0005 | 0.9999 | 0.0031 | 0.5968 | 0.0002 |
| 2.1 | 0.0004 | 0.7619 | 0.0018 | 0.9998 | 0.0091 | 0.6832 | 0.0008 |

**Table S4. ANOVA and Tukey's post-hoc analysis of photopic b-wave data in Fig. 1E**

| Flash intensity (log cd·s/m$^2$) | *p*-value | | | | | | |
|---|---|---|---|---|---|---|---|
| | ANOVA | Control vs DHA-adq | Control vs DHA-def | Control vs DHA-rep | DHA-adq vs DHA-def | DHA-adq vs DHA-rep | DHA-def vs DHA-rep |
| -1.0 | 0.2713 | 0.5989 | 0.2054 | 0.6389 | 0.8501 | 0.9999 | 0.8144 |
| -0.4 | < 0.0001 | 0.9066 | < 0.0001 | 0.1027 | < 0.0001 | 0.2618 | < 0.0001 |
| 0.2 | 0.0009 | 0.6372 | 0.0011 | 0.4922 | 0.0086 | 0.9935 | 0.0149 |
| 0.8 | 0.0001 | 0.9710 | 0.0005 | 0.8865 | 0.0006 | 0.9900 | 0.0011 |
| 1.4 | 0.0006 | 0.8586 | 0.0016 | 0.9620 | 0.0052 | 0.9877 | 0.0026 |
| 2.0 | 0.0001 | 0.8943 | 0.0003 | 0.8343 | 0.0007 | 0.9988 | 0.0010 |

**Table S5. ANOVA and Tukey's post-hoc analysis of ERG parameters in Table 2**

| ERG parameter | *p*-value | | | | | | |
|---|---|---|---|---|---|---|---|
| | ANOVA | Control vs DHA-adq | Control vs DHA-def | Control vs DHA-rep | DHA-adq vs DHA-def | DHA-adq vs DHA-rep | DHA-def vs DHA-rep |
| $S$ | 0.0015 | 0.237 | 0.0008 | 0.1118 | 0.0371 | 0.9642 | 0.0891 |
| $Rm_{P3}$ | 0.2325 | 0.995 | 0.3949 | 0.9816 | 0.5308 | 0.927 | 0.2284 |
| $t_d$ | 0.0025 | 0.1156 | 0.0018 | 0.4987 | 0.1741 | 0.745 | 0.0274 |
| Scotopic $K$ | 0.1256 | 0.9738 | 0.2798 | 0.6616 | 0.1411 | 0.4222 | 0.9079 |
| Scotopic $R_{max}$ | < 0.0001 | > 0.9999 | < 0.0001 | 0.1563 | < 0.0001 | 0.1643 | < 0.0001 |
| Scotopic $n$ | 0.0019 | 0.9602 | 0.0073 | 0.1966 | 0.0029 | 0.088 | 0.3384 |
| Photopic $K$ | 0.2908 | > 0.9999 | 0.3753 | 0.9992 | 0.3944 | 0.9997 | 0.4447 |
| Photopic $R_{max}$ | 0.0222 | 0.9916 | 0.0291 | 0.8399 | 0.0500 | 0.9465 | 0.1344 |
| Photopic $n$ | 0.1371 | 0.4649 | 0.101 | 0.366 | 0.7786 | 0.9974 | 0.8758 |

**Table S6. ANOVA and Tukey's post-hoc analysis of c-wave data in Fig. 1F**

| *p*-value | | | | | | |
|---|---|---|---|---|---|---|
| ANOVA | Control vs DHA-adq | Control vs DHA-def | Control vs DHA-rep | DHA-adq vs DHA-def | DHA-adq vs DHA-rep | DHA-def vs DHA-rep |
| 0.0135 | 0.9654 | 0.0162 | 0.4413 | 0.0421 | 0.7097 | 0.3043 |

**Table S7. ANOVA and Tukey's post-hoc analysis of histological data in Figs. 2B and 2C**

| Histological parameter | *p*-value | | | |
|---|---|---|---|---|
| | ANOVA | Control vs DHA-adq | Control vs DHA-def | DHA-adq vs DHA-def |
| Number of nuclei | 0.9465 | 0.9816 | 0.9414 | 0.9881 |
| ROS length | < 0.0001 | 0.9221 | < 0.0001 | < 0.0001 |

**Table S8. T-test analysis of AFM data from ROS disc membranes prepared and analyzed by different users**

| ROS disc membrane property | Set 1 [a] (*n*=80) | Set 2 [b] (*n*=95) | *p*-value |
|---|---|---|---|
| Disc diameter (μm) | 1.23 ± 0.31 | 1.26 ± 0.30 | 0.4663 |
| Mean nanodomain size ($nm^2$) | 1360 ± 389 | 1281 ± 307 | 0.1393 |
| Median nanodomain size ($nm^2$) | 959 ± 278 | 920 ± 195 | 0.2788 |
| Number of nanodomains | 154 ± 98 | 156 ± 103 | 0.8918 |
| Number of rhodopsin | 14774 ± 10200 | 14105 ± 9882 | 0.6605 |
| Nanodomain density ($\mu m^{-2}$) | 215 ± 67 | 234 ± 65 | 0.0566 |
| Rhodopsin density ($\mu m^{-2}$) | 20053 ± 6188 | 20661 ± 5381 | 0.4875 |

[a] Data are those reported previously in Rakshit and Park (2015) *Biochemistry 54*, 2885-2894.
[b] Data are from the current study.

**Table S9. ANOVA and Tukey's post-hoc analysis of ROS disc membrane properties in Table 3**

| ROS disc membrane property | *p*-value | | | | | | |
|---|---|---|---|---|---|---|---|
| | ANOVA | Control vs DHA-adq | Control vs DHA-def | Control vs DHA-rep | DHA-adq vs DHA-def | DHA-adq vs DHA-rep | DHA-def vs DHA-rep |
| Disc diameter | 0.7731 | 0.9982 | 0.9046 | 0.9123 | 0.8422 | 0.8581 | 0.9999 |
| Mean nanodomain size | 0.6048 | 0.9987 | 0.6953 | 0.9661 | 0.6289 | 0.9353 | 0.9594 |
| Median nanodomain size | 0.0506 | 0.0404 | 0.9451 | 0.8659 | 0.1228 | 0.3400 | 0.9909 |
| Number of nanodomains | 0.0002 | 0.9994 | 0.0047 | 0.9831 | 0.0053 | 0.9670 | 0.0430 |
| Number of rhodopsin | 0.0009 | 0.9993 | 0.0035 | 0.9733 | 0.0039 | 0.9521 | 0.0419 |
| Nanodomain density | < 0.0001 | 0.8240 | < 0.0001 | 0.8895 | < 0.0001 | 0.4524 | < 0.0001 |
| Rhodopsin density | < 0.0001 | 0.9075 | < 0.0001 | 0.6336 | < 0.0001 | 0.2955 | < 0.0001 |

**Table S10. ANOVA and Tukey's post-hoc analysis of 11-*cis* retinal quantification in Table 4**

| *p*-value | | | |
|---|---|---|---|
| ANOVA | Control vs DHA-adq | Control vs DHA-def | DHA-adq vs DHA-def |
| 0.0809 | 0.9108 | 0.0778 | 0.2217 |

**Table S11. T-test analysis of ROS disc membrane properties of DHA-deficient and *Gnat*$^{-/-}$ mice**

| ROS disc membrane property | DHA-deficient [a] (*n*=108) | G*nat*$^{-/-}$ [b] (*n*=107) | *p*-value |
|---|---|---|---|
| Disc diameter (μm) | 1.30 ± 0.39 | 1.34 ± 0.45 | 0.4378 |
| Mean nanodomain size ($nm^2$) | 1331 ± 319 | 1332 ± 292 | 0.9745 |
| Median nanodomain size ($nm^2$) | 903 ± 230 | 804 ± 178 | 0.0005 |
| Number of nanodomains | 212 ± 150 | 220 ± 141 | 0.6936 |
| Number of rhodopsin | 19619 ± 14205 | 20968 ± 14327 | 0.4889 |
| Nanodomain density ($\mu m^{-2}$) | 313 ± 80 | 308 ± 74 | 0.6404 |
| Rhodopsin density ($\mu m^{-2}$) | 28259 ± 4416 | 28254 ± 5316 | 0.9939 |

[a] Data are from the current study.
[b] Data are those reported previously in Rakshit et al. (2017) *Biochim. Biophys. Acta 1864*, 1691-1702.