Supplemental File for

*Change in Blood and Benign Breast Biomarkers in Women Undergoing a Weight Loss Intervention Randomized to High Dose ω-3 Fatty Acids vs Placebo*.

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Supplemental Table 1: Specimen collection and assay methods details.

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Supplemental Table 4. Median relative difference (percent) between baseline and 12 months for 19 serum biomarkers (of a total of 24 assessed) which exhibited a statistically significant change over time\* either for the total cohort of 35 women completing the 12-month trial and/or for groups defined by randomization or by dichotomization at 10% weight loss achieved at 6 months.

Supplemental Table 5: Biomarker change at 12 months by subgroups defined by randomization arm (placebo vs ω-3 FA) and dichotomization by weight loss (<10% or >10%). Biomarkers are grouped into categories of adipokines, cytokines, hormones/growth factors, and insulin. A total of 24 biomarkers or ratios were assessed at 12 months.

Supplemental Table 6: Levels and changes for adiponectin assessed in serum collected both fasting and non-fasting, and in benign breast tissue acquired non-fasting by RPFNA.

Supplemental Table 7: Baseline, 6-month, and 12-month values and change over time for Ki-67, Masood cytomorphology score.

**Supplemental Table 1**: Specimen collection and assay methods details.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Collection Condition and Specimen | | | **Biomarker** | **Method of Analysis** | **Source & Product Number** |
| **Fasting**  **Serum** | **Non-Fasting**  **Serum** | **Non-Fasting Tissue Lysate** |
|  |  | X | Interleukin-8 (IL-8) (tissue only) | Luminex:  Milliplex® Human Adipokine Magnetic Bead Panels 1&2 for serum  Milliplex® MAP Human Adipocyte Magnetic Bead Panel for tissue | Millipore-Sigma: HADK1MAG-61K & HADK2MAG-61K for serum  Millipore-Sigma: HADCYMAG-61K for tissue |
| X | X | Adiponectin |
| Hepatocyte growth factor (HGF) |
| Interleukin-6 (IL-6) |
| Leptin |
| Macrophage Chemotactic Protein 1 (MCP-1) |
| PAI-1 |
| Resistin |
| Tumor Necrosis Factor-α (TNF α) |
|  | Insulin (serum only) |
|  | Lipocalin-2 (serum only) |
|  |  | Fatty Acid Binding Protein 4 (FABP4) | ELISA | Cayman: 10007614 |
|  |  | Fibroblast Growth Factor 21 (FGF-21) baseline & 6 month only | R&D Systems: DF2100 |
|  |  | Omentin-1 | RayBiotech EIA-OME |
|  | X |  | C-Reactive Protein (CRP) | Diagnostics Biochem Canada: CAN-CRP-4360 |
|  |  | Estradiol | Diagnostics Biochem Canada: CAN-E- 430 |
|  |  | Testosterone | Diagnostics Biochem Canada: CAN-TE-250 |
|  |  | SHBG | Diagnostics Biochem Canada: CAN-SHBG-410 |
|  |  | Insulin like Growth Factor-1 (IGF-1) | Milliplex® MAP Magnetic Bead Panels | Millipore-Sigma: HIGFMAG-52K |
|  |  | Insulin-like Growth Factor Binding Protein 2 (IGFBP2) | Millipore-Sigma: HIGFMAG-53K |
|  |  | Insulin-like Growth Factor Binding Protein 3 (IGFBP3) |

**Supplemental Table 2 Adverse Events**

Over the entire 12 months of the study, a total of 182 adverse events (AEs) and 2 serious adverse events (SAEs) were self-reported and recorded in the database from a total of 43 subjects; 3 subjects failed to report a single AE.

Number and worst grade AE reported by randomization arm:

|  | Initial 6 months | | Second 6 months | | Entire 12 months | |
| --- | --- | --- | --- | --- | --- | --- |
| Worst AE Reported | Placebo  (N=23) | ω-3 FA  (N=23) | Placebo  (N=20) | ω-3 FA  (N=19) | Placebo  (N=23) | ω-3 FA (N=23) |
| Grade 0 | 4 | 3 | 9 | 5 | 3 | 1 |
| Grade 1 | 5 | 5 | 5 | 4 | 4 | 3 |
| Grade 2 | 13 | 14 | 6 | 6 | 15 | 14 |
| Grade 3 | 1 | 1 | 0 | 2 | 1 | 3 |
| SAE | 0 | 0 | 0 | 2 | 0 | 2 |

There was no statistically significant difference between the groups however caution is advised in interpretation as no adjustment was made for multiple comparisons.

The two SAEs, both in subjects randomized to receive omega-3 fatty acid supplementation, were diagnoses of DCIS and invasive lobular carcinoma that required hospitalization. The diagnosis of DCIS (subject 614) occurred 2 days after the second RPFNA (i.e., after 6 months of supplementation). The diagnosis of lobular invasive carcinoma (subject 633) occurred 7 days after the third RPFNA (*i.e.*, after 12 months of supplementation). Neither event is considered to have been related to the study intervention; rather to the high risk for development of breast cancer which was an eligibility criterion. Excluding the two subjects with SAEs, a total of four Grade 3 events were reported by four subjects. These are detailed below, along with the randomization arm.

Grade 3 AEs by subject:

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Study ID | Adverse Event | Onset from Start of Agent | Duration | Relationship |
| 617 | Vomiting | 187 d after ω-3 FA | 2 d | Not related |
| 618 | Anaphylaxis | 342 d after ω-3 FAv | 1 d | Not related |
| 635 | Oral pain | 57 d after Placebo | 34 d | Not related |
| 664 | Aspiration | 281 d after ω-3 FA | 39 d | Not related |

**Supplemental Table 3**. Value and changes in ratio of (DHA+EPA)/AA in erythrocyte phospholipids.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Timepoint or Change | Placebo | | Omega-3 Fatty Acid Supplementation | |
|  | N | Median (range) | N | Median (range) |
| a Baseline | 18 | 0.31(0.20 – 0.51) | 21 | 0.31 (0.23 – 0.49) |
| 6 months | 20 | 0.30 (0.21 – 0.42) | 19 | 0.76 (0.59 – 1.08) |
| Change, baseline to 6 months | 16 | 0.00 (-0.08 – 0.08) | 17 | 0.45 (0.26 – 0.80) |
| Relative change (%), 0 to 6 months | 16 | 0 (-20 – 24) | 17 | 156 (77 – 282) |
| Ratio 6-months: baseline | 16 | 1.00 (0.80 – 1.24) | 17 | 2.56 (1.77 – 3.82) |
| 12 months | 19 | 0.29 (0.18 – 0.40) | 16 | 0.84 (0.34 – 1.15) |
| Change, baseline to 12 months | 16 | -0.02 (-0.11 – 0.03) | 14 | 0.52 (0.28 – 0.89) |
| Relative change (%), 0 to 12 months | 16 | -9 (-28 – 11) | 14 | 165 (81 – 286) |
| Ratio 12-months: baseline | 16 | 0.91 (0.72 – 1.11) | 14 | 2.65 (1.81 – 4.67) |

Statistically significant differences between groups (*P* < 0.001; Mann-Whitney non-parametric test) for all except baseline values (a*P* = 0.91). For within group changes over time (Wilcoxon non-parametric test), the Supplementation group exhibited statistically significant increases from baseline to 6 months (*P* < 0.001) and baseline to 12 months (*P* = 0.001); but no change between 6 and 12 months (*P* = 0.14). For the placebo group there were no significant changes from baseline to 6 months (*P* = 0.72) but there was a statistically significant decrease between baseline and 12 months (*P* = 0.008). Caution is advised in interpretation of results as there was no adjustment made for multiple comparisons.

**Supplemental Table 4.** Median relative difference (percent) between baseline and 12 months for 19 serum biomarkers (of a total of 24 assessed) which exhibited a statistically significant change over time\* either for the total cohort of 35 women completing the 12-month trial and/or for groups defined by randomization or by dichotomization at 10% weight loss achieved at 6 months.

| Analyte or Ratio | *Relative Difference (percent)* | | | | |
| --- | --- | --- | --- | --- | --- |
| Total Cohort | Placebo | ω-3 FA | Wt Loss  <10% | Wt loss  > 10% |
| N=35 | N=19 | N=16 | N=17 | N=18 |
| Adiponectin | **39%\*\*\*** | **27%\*** | **45%\*** | **20%\*** | **50%\*\*** |
| Leptin | **-37%\*\*\*** | **-46%\*\*** | **-17%\*** | -3% | **-61%\*\*\*** |
| Adiponectin:Leptin Ratio † | **103%\*\*\*** | **113%\*\*\*** | **54%\*\*** | **22%\*** | **312%\*\*\*** |
| Lipocalin-2 | **-20%\*\*** | **-14%\*** | **-24%\*** | **-17%\*** | **-20%\*\*** |
| Resistin | **-14%\*\*\*** | **-6%\*** | **-18%\*\*** | -5**%** | **-14%\*\*\*** |
| PAI-1 | **-12%\*\*\*** | **-11%\*** | **-13%\*\*** | **-6%\*** | **-19%\*\*** |
| HGF | **-20%\*\*\*** | **-20%\*\*** | **-23%\*\*** | **-15%\*** | **-22%\*\*\*** |
| Omentin | 14% | 6**%** | **18%\*** | 9**%** | 18**%** |
| Insulin | **-40%\*** | -40**%** | **-42%\*** | -15**%** | **-53%\*** |
| CRP | **-40%\*\*\*** | **-25%\*** | **-23%\*** | **-13%\*** | **-64%\*\*** |
| IL-6 | **-33%\*\*** | **-37%\*** | **-28%\*** | **-34%\*** | 19**%** |
| MCP-1 | **-9%\*** | -16% | -4% | -5% | -13% |
| TNF-α | **-7%\*** | -10% | -4% | -4% | -10% |
| FABP4 | **8%\*** | **-12%\*** | -3% | -6% | -12% |
| SHBG | **9%\*\*\*** | 7% | **23%\*\*** | **3%\*\*** | **38%\*\*\*** |
| Estradiol | 10% | 16% | 7% | **12%\*** | 7% |
| IGF1 | **11%\*** | -2% | **23%\*** | 7% | 21% |
| IGFBP2 (N=18) | 34% | **74%\*** | 5**%** | 5% | **106%\*** |
| IGF1:IGFBP3 ratio | **16%\*** | 6% | **33%\*** | 16% | 17% |

\* Wilcoxon non-parametric signed-rank test, 2-tailed, for within-group differences between baseline and 12 months. There is no adjustment for multiple comparisons so caution is advised in interpretation of the results. *P*-values: \*, <0.05; \*\*, <0.005; \*\*\*, <0.0005.

† Adiponectin:Leptin Ratio is computed on the basis of adiponectin values in μg/ml and leptin values in ng/ml.

Also assessed were testosterone, bioavailable estradiol, bioavailable testosterone, IGFBP3, IGF1:IGBP2 ratio; no statistically significant changes detected.

**Supplemental Table 5**: Biomarker change at 12 months by subgroups defined by randomization arm (placebo vs ω-3 FA) and dichotomization by weight loss (<10% or >10%). Biomarkers are grouped into categories of adipokines, cytokines, hormones/growth factors, and insulin. A total of 24 biomarkers or ratios were assessed at 12 months.

| Biomarker or Ratio | Median relative change and associated *P*-value for change over time | | | | | | | |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| <10% weight loss  Placebo  Subgroup 1 N=8 | | <10% weight loss  ω-3 FA  Subgroup 2 N=9 | | >10% weight loss Placebo  Subgroup 3 N=11 | | >10% Weight loss  ω-3 FA  Subgroup 4 N=7 | |
| Adiponectin | 33% | 0.05 | 0% | 0.44 | 27% | 0.091 | 77% | **0.018** |
| Leptin | -2% | 0.67 | -3% | 0.59 | -67% | **0.0034** | -56% | **0.028** |
| Adiponectin:Leptin Ratio | 35% | 0.05 | 14% | 0.086 | 231% | **0.0034** | 481% | **0.018** |
| Lipocalin-2 | -12% | 0.40 | -29% | 0.051 | -20% | **0.016** | -20% | 0.063 |
| Resistin | -3% | 0.44 | -18% | **0.038** | -9% | **0.0051** | -17% | **0.034** |
| PAI-1 | -4% | 0.40 | -9% | 0.058 | -17% | **0.033** | -23% | **0.028** |
| HGF | -13% | 0.40 | -15% | **0.028** | -21% | **0.0034** | -32% | 0.063 |
| Omentin | -8% | 0.40 | 16% | 0.051 | 12% | 0.51 | 19% | 0.18 |
| CRP | -13% | 0.16 | -32% | 0.17 | -56% | 0.051 | -70% | **0.018** |
| IL6 | -37% | **0.036** | -32% | 0.14 | -32% | 0.35 | -19% | 0.091 |
| TNF-α | -9% | 0.093 | -2% | 0.95 | -15% | 0.72 | -10% | 0.051 |
| SHBG | -3% | 0.26 | 5% | 0.051 | 36% | **0.0059** | 77% | **0.018** |
| Estradiol | 32% | **0.012** | 7% | 1.00 | 10% | 0.80 | -15% | 1.00 |
| Bioavailable Estradiol | 44% | **0.035** | 0% | 1.00 | -4% | 0.61 | -46% | 0.31 |
| IGF1 | 2% | 0.57 | 21% | 0.21 | -2% | 0.93 | 45% | **0.043** |
| IGFBP3:IGF1 ratio | 15% | 0.33 | 22% | 0.14 | -2% | 0.93 | 37% | **0.043** |
| Insulin | -7% | 0.67 | -15% | 0.21 | -48% | 0.075 | -53% | **0.043** |
| Number Modulated | **5** | | **2** | | **7** | | **10** | |

\* Wilcoxon non-parametric signed-rank test, 2-tailed, for within-subgroup differences between baseline and 12 months.

There is no adjustment for multiple comparisons so caution is advised in interpretation of the results.

**Supplemental Table 6:** Levels and changes for adiponectin assessed in serum collected both fasting and non-fasting, and in benign breast tissue acquired non-fasting by RPFNA.

| Adiponectin | <10% weight loss  Placebo | <10% weight loss  ω-3 FA | >10% weight loss Placebo | >10% Weight loss  ω-3 FA | Differences Between groups  (P-values by  Kruskal Wallis test followed by individual  Mann–Whitney tests) |
| --- | --- | --- | --- | --- | --- |
|  | Group 1 N=11 | Group 2 N=10 | Group 3 N=11 | Group 4 N=10 |
| **Serum Assessment (ng/ml) Fasting** | | | | | |
| Baseline | 19.1 | 27.9 | 34.6 | 29.3 | 0.51 |
| 6-month | 26.3 | 25.3 | 48.9 | 31.1 | 0.23  2 vs 3: 0.041 |
| Baseline to 6-month change | 0.5 | 3.2 | 6.7 | 4.9 | 0.36 |
| Baseline to 6-month % change | 1.6 | 10.7 | 19.1 | **29.9** | 0.71 |
| 12-month | 47.1 | 28.9 | 44.3 | 53.9 | 0.51 |
| Baseline to 12-month change | 11.2 | -0.05 | 9.4 | 21.1 | 0.044  2 vs 4: 0.007  3 vs 4: 0.033 |
| Baseline to 12-month % change | 32.7 | 0 | 27.3 | **77.1** | 0.017  1 vs 4: 0.040  2 vs 4: 0.007  3 vs 4: 0.010 |
| **Serum Assessment (ng/ml) Non-Fasting** | | | | | |
| Baseline | 20.4 | 25.5 | 32.9 | 27.9 | 0.68 |
| 6-month | 24.3 | 23.8 | 38.6 | 32.6 | 0.29 |
| Baseline to 6-month change | 2.3 | 2.0 | 6.3 | 8.5 | 0.28 |
| Baseline to 6-month % change | 7 | 7 | 30 | **42** | 0.10  1 vs 4: 0.049  2 vs 4: 0.041 |
| 12-month | 44.7 | 28.8 | 43.7 | 53.7 | 0.30  2 vs 3: 0.044 |
| Baseline to 12-month change | 6.8 | 1.9 | 7.2 | 21.2 | 0.062  2 vs 4: 0.004 |
| Baseline to 12-month % change | 43 | 5 | 46 | **125** | 0.015  1 vs 4: 0.028  2 vs 4: 0.004  3 vs 4: 0.042 |
| **Tissue Assessment (pg/µg protein) Non-Fasting** | | | | | |
| Baseline | 208 | 276 | 379 | 214 | 0.32 |
| 6-month | 233 | 216 | 600 | 207 | 0.22 |
| Baseline to 6-month change | -11 | -5 | 0 | 18 | 0.93 |
| Baseline to 6-month % change | -7 | -10 | 0 | 13 | 0.89 |
| 12-month | 187 | 225 | 440 | 472 | 0.13 |
| Baseline to 12-month change | -75 | -12 | 75 | 296 | 0.093  1 vs 4: 0.015  2 vs 4: 0.050 |
| Baseline to 12-month % change | -24 | -4 | 20 | **134** | 0.024  1 vs 4: 0.003  2 vs 4: 0.017 |

**Bold** indicates a statistically significant change over time within group 4 by Wilcoxon nonparametric signed-rank test. There is no adjustment for multiple comparisons so caution is advised in interpretation of the results.

**Supplemental Table 7:** Baseline, 6-month, and 12-month values and change over time for Ki-67, Masood cytomorphology score.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Variable and Time/Change | Median (range) | | | |
| Placebo  N=22  N=19 (12 mo) | ω-3 FA  N=20  N=16 (12 mo) | <10% Wt loss  N=21  N=17 (12 mo) | >10% Wt loss  N=21  N=18 (12 mo) |
| Ki-67 (% positively stained cells by immunocytochemistry) | | | | |
| Baseline | 0.7  (0 – 19.8) | 0.7  (0 – 7.2) | 0.8  (0 – 11.4) | 0.6  (0 – 19.8) |
| 6-month | 1.3  (0 – 23.2) | 1.3  (0 – 10.4) | 1.8  (0 – 15.0) | 0.4  (0 – 23.2) |
| Change over time (0 to 6 mo) | 0.4  (-4.4 – 10.0) | 0.0  (-2.8 – 5.8) | 0.9  (-4.4 – 10.0) | -0.2  (-2.8 – 8.0) |
| Within group (Wilcoxon test) | 0.12 | 0.35 | 0.020 | 0.68 |
| 12-month | 1.2  (0 – 14.0) | 1.3  (0 – 8.8) | 1.4  (0 – 8.8) | 1.  (0 – 14.0) |
| Change over time (0 to 12 mo) | 0.2  (-9.4 – 13.4) | 0.0  (-3.2 – 1.8) | 0.6  (-6.8 – 4.3) | -0.1  (-9.4 – 13.4) |
| Within group (Wilcoxon test) | 0.38 | 0.82 | 0.20 | 0.62 |
| Masood Score | | | | |
| Baseline | 13  (12 – 15) | 14  (12 – 16) | 13  (12 – 16) | 14  (12 – 15) |
| 6-month | 13  (9 – 16) | 13  (11 – 15) | 13  (11 – 16) | 13  (9 – 16) |
| Change over time (0 to 6 mo) | 0  (-6 – 3) | 0  (-2 – 2) | 0  (-2 – 2) | 0  (-6 – 3) |
| Within group (Wilcoxon test) | 0.95 | 0.36 | 0.82 | 0.57 |
| 12-month | 13  (9 – 15) | 13  (10 – 15) | 13  (10 –15) | 13  (9 – 15) |
| Change over time (0 to 12 mo) | 0  (-6 – 3) | -1  (-3 – 2) | 0  (-3 – 3) | -1  (-6 – 1) |
| Within group (Wilcoxon test) | 0.23 | 0.11 | 0.75 | **0.009** |

There is no adjustment for multiple comparisons so caution is advised in interpretation of the results.