# Supplementary tables and experimental design for EPA and CAP interactions in adipocytes and breast cancer cell crosstalk

| Table 1: Exploratory factorial regression analyses to examine CAP and EPA interactions of mRNA markers associated with MDA-MB-231 cell growth and inflammation | | | | | |
|---|---|---|---|---|---|
| Marker | IVs | Betas | SE | T-value | p-value |
| FASN | Intercept | 1 | 0.13677 | 7.311526 | 3.84E-08 |
| | CM | 4.659069 | 0.205156 | 22.70994 | 1.88E-20 |
| | EPA | -0.33801 | 0.193422 | -1.74751 | 0.090779 |
| | CAP | -0.14089 | 0.193422 | -0.7284 | 0.472019 |
| | CM:EPA | -4.73081 | 0.281959 | -16.7784 | 8.61E-17 |
| | CM:CAP | -4.41591 | 0.281959 | -15.6615 | 5.54E-16 |
| | EPA:CAP | -0.04515 | 0.281959 | -0.16011 | 0.873865 |
| | **CM:EPA:CAP** | **4.482303** | **0.398751** | **11.24087** | **2.80E-12** |

Multiple R-squared: 0.969194; F-statistic: 134.835on 7 and 30 degrees of freedom

| Table 2: Exploratory factorial regression analyses to examine CAP and EPA interactions of mRNA markers associated with MDA-MB-231 cell growth and inflammation | | | | | |
|---|---|---|---|---|---|
| Marker | IVs | Betas | SE | T-value | p-value |
| STAT3 | Intercept | 1 | 0.089959 | 11.11617 | 2.41E-12 |
| | CM | 2.182367 | 0.134939 | 16.17304 | 1.16E-16 |
| | EPA | -0.0585 | 0.127221 | -0.45983 | 0.648844 |
| | CAP | -0.11335 | 0.127221 | -0.89098 | 0.379801 |
| | CM:EPA | -2.13889 | 0.185455 | -11.5332 | 9.57E-13 |
| | CM:CAP | -2.0826 | 0.185455 | -11.2296 | 1.87E-12 |
| | EPA:CAP | 0.162369 | 0.179918 | 0.90246 | 0.373771 |
| | **CM:EPA:CAP** | **1.760098** | **0.258388** | **6.811848** | **1.25E-07** |

Multiple R-squared: 0.936673; F-statistic: 65.50367 on 7 and 31 degrees of freedom

| Table 3: Exploratory factorial regression analyses to examine CAP and EPA interactions of mRNA markers associated with MDA-MB-231 cell growth and inflammation | | | | | |
|---|---|---|---|---|---|
| Marker | IVs | Betas | SE | T-value | p-value |
| NF-κB | Intercept | 1 | 0.085336 | 11.7184 | 6.38E-13 |
| | CM | 0.77946 | 0.128004 | 6.089348 | 9.52E-07 |
| | EPA | -0.24332 | 0.120683 | -2.01618 | 0.052517 |
| | CAP | -0.12927 | 0.120683 | -1.07112 | 0.292383 |
| | CM:EPA | -0.46442 | 0.175924 | -2.63989 | 0.012866 |
| | CM:CAP | -0.61338 | 0.175924 | -3.4866 | 0.001485 |
| | EPA:CAP | 0.147165 | 0.170672 | 0.862269 | 0.395161 |
| | CM:EPA:CAP | 0.28808 | 0.245109 | 1.175314 | 0.248818 |

Multiple R-squared: 0.748958; F-statistic: 13.21216 on 7 and 31 degrees of freedom

**Supplementary tables and experimental design for EPA and CAP interactions in adipocytes and breast cancer cell crosstalk**

| Table 4: Exploratory factorial regression analyses to examine CAP and EPA interactions of mRNA markers associated with MDA-MB-231 cell growth and inflammation | | | | | |
|---|---|---|---|---|---|
| Marker | IVs | Betas | SE | T-value | p-value |
| IL-6 | Intercept | 1 | 0.080806 | 12.37536 | 9.70E-14 |
| | CM | 8.653 | 0.127765 | 67.72585 | 3.96E-36 |
| | EPA | -0.5338 | 0.119854 | -4.4537 | 9.64E-05 |
| | CAP | -0.03896 | 0.119854 | -0.32504 | 0.747265 |
| | CM:EPA | -8.23044 | 0.178871 | -46.0132 | 8.22E-31 |
| | CM:CAP | -8.60377 | 0.184265 | -46.6923 | 5.18E-31 |
| | EPA:CAP | 0.034969 | 0.1695 | 0.206305 | 0.83786 |
| | **CM:EPA:CAP** | **7.800978** | **0.252962** | **30.83854** | **2.26E-25** |

Multiple R-squared: 0.995743; F-statistic: 1069.25 on 7 and 32 degrees of freedom

| Table 5: Exploratory factorial regression analyses to examine CAP and EPA interactions of mRNA markers associated with MDA-MB-231 cell growth and inflammation | | | | | |
|---|---|---|---|---|---|
| Marker | IVs | Betas | SE | T-value | p-value |
| IL-8 | Intercept | 1 | 0.037364 | 26.76375 | 5.92E-24 |
| | CM | 0.86025 | 0.059078 | 14.56135 | 6.34E-16 |
| | EPA | -0.66817 | 0.05542 | -12.0565 | 1.23E-13 |
| | CAP | -0.01818 | 0.05542 | -0.32803 | 0.74496 |
| | CM:EPA | -0.18147 | 0.082709 | -2.19414 | 0.035373 |
| | CM:CAP | -0.69187 | 0.082709 | -8.36508 | 1.16E-09 |
| | EPA:CAP | 0.029101 | 0.078375 | 0.371298 | 0.712789 |
| | **CM:EPA:CAP** | **0.473915** | **0.115164** | **4.115145** | **0.000242** |

Multiple R-squared: 0.965257; F-statistic: 130.975 on 7 and 33 degrees of freedom

| Table 6: Exploratory factorial regression analyses to examine CAP and EPA interactions of protein markers associated with MDA-MB-231 cell inflammation | | | | | |
|---|---|---|---|---|---|
| Marker | IVs | Betas | SE | T-value | p-value |
| IL-6 | Intercept | 1 | 0.671968 | 1.488166 | 0.162508 |
| | CM | 9.992979 | 1.062475 | 9.40538 | 6.92E-07 |
| | EPA | -0.48198 | 0.950306 | -0.50718 | 0.621221 |
| | CAP | 0.009325 | 0.950306 | 0.009813 | 0.992332 |
| | CM:EPA | -9.8201 | 1.502566 | -6.53556 | 2.79E-05 |
| | CM:CAP | -10.2927 | 1.502566 | -6.85011 | 1.77E-05 |
| | EPA:CAP | -0.00559 | 1.343936 | -0.00416 | 0.996752 |
| | **CM:EPA:CAP** | **10.19462** | **2.124949** | **4.797581** | **0.000435** |

Multiple R-squared: 0.92132; F-statistic: 20.07372 on 7 and 12 degrees of freedom

# Supplementary tables and experimental design for EPA and CAP interactions in adipocytes and breast cancer cell crosstalk

| Table 7: Exploratory factorial regression analyses to examine CAP and EPA interactions of protein markers associated with MDA-MB-231 cell inflammation | | | | | |
|---|---|---|---|---|---|
| Marker | IVs | Betas | SE | T-value | p-value |
| IL-8 | Intercept | 1 | 0.611433 | 1.635502 | 0.122749 |
| | CM | 7.91525 | 0.864697 | 9.153783 | 1.58E-07 |
| | EPA | -0.48738 | 0.864697 | -0.56364 | 0.581328 |
| | CAP | -0.0027 | 0.864697 | -0.00313 | 0.997547 |
| | CM:EPA | -8.17419 | 1.297046 | -6.30216 | 1.42E-05 |
| | CM:CAP | -8.43167 | 1.222866 | -6.895 | 5.10E-06 |
| | EPA:CAP | 0.017865 | 1.222866 | 0.014609 | 0.988537 |
| | **CM:EPA:CAP** | **8.483555** | **1.782619** | **4.75904** | **0.000253** |

Multiple R-squared: 0.915312; F-statistic: 23.16021 on 7 and 15 degrees of freedom

| Table 8: Exploratory factorial regression analyses to examine CAP and EPA interactions of mRNA markers associated with MCF-7 cell growth and inflammation | | | | | |
|---|---|---|---|---|---|
| Marker | IVs | Betas | SE | T-value | p-value |
| FASN | Intercept | 1 | 0.219363 | 4.558658 | 4.40E-05 |
| | CM | 5.041204 | 0.322893 | 15.6126 | 4.30E-19 |
| | EPA | -0.10044 | 0.322893 | -0.31107 | 0.757287 |
| | CAP | -0.11523 | 0.310226 | -0.37144 | 0.712179 |
| | CM:EPA | -5.18877 | 0.465339 | -11.1505 | 3.94E-14 |
| | CM:CAP | -5.13108 | 0.45664 | -11.2366 | 3.09E-14 |
| | EPA:CAP | -0.07219 | 0.45664 | -0.15809 | 0.875146 |
| | **CM:EPA:CAP** | **5.201977** | **0.658088** | **7.904679** | **7.61E-10** |

Multiple R-squared: 0.910932; F-statistic: 61.36463 on 7 and 42 degrees of freedom

| Table 9: Exploratory factorial regression analyses to examine CAP and EPA interactions of mRNA markers associated with MCF-7 cell growth and inflammation | | | | | |
|---|---|---|---|---|---|
| Marker | IVs | Betas | SE | T-value | p-value |
| STAT3 | Intercept | 1 | 0.152655 | 6.550723 | 2.79E-08 |
| | CM | 1.281499 | 0.230793 | 5.552603 | 1.02E-06 |
| | EPA | -0.0918 | 0.222531 | -0.41255 | 0.681667 |
| | CAP | -0.17619 | 0.215887 | -0.81614 | 0.418213 |
| | CM:EPA | -1.07134 | 0.338285 | -3.16697 | 0.002601 |
| | CM:CAP | -1.19757 | 0.32639 | -3.66914 | 0.000582 |
| | EPA:CAP | 0.029257 | 0.310044 | 0.094363 | 0.925191 |
| | **CM:EPA:CAP** | **1.166133** | **0.493395** | **2.363488** | **0.021953** |

Multiple R-squared: 0.532763; F-statistic: 8.307471 on 7 and 51 degrees of freedom

## Supplementary tables and experimental design for EPA and CAP interactions in adipocytes and breast cancer cell crosstalk

| Table 10: Exploratory factorial regression analyses to examine CAP and EPA interactions of mRNA markers associated with MCF-7 cell growth and inflammation | | | | | |
|---|---|---|---|---|---|
| Marker | IVs | Betas | SE | T-value | p-value |
| NF-κB | Intercept | 1 | 0.101256 | 9.876006 | 1.97E-13 |
| | CM | 2.097104 | 0.160099 | 13.09879 | 6.06E-18 |
| | EPA | -0.08165 | 0.147604 | -0.55319 | 0.582552 |
| | CAP | -0.13708 | 0.143197 | -0.95731 | 0.342925 |
| | CM:EPA | -2.0176 | 0.229227 | -8.80176 | 8.30E-12 |
| | CM:CAP | -2.09184 | 0.226414 | -9.239 | 1.78E-12 |
| | EPA:CAP | 0.018441 | 0.205651 | 0.08967 | 0.928901 |
| | **CM:EPA:CAP** | **2.086325** | **0.322193** | **6.475388** | **3.67E-08** |

Multiple R-squared: 0.848026; F-statistic: 40.6547 on 7 and 51 degrees of freedom

| Table 11: Exploratory factorial regression analyses to examine CAP and EPA interactions of mRNA markers associated with MCF-7 cell growth and inflammation | | | | | |
|---|---|---|---|---|---|
| Marker | IVs | Betas | SE | T-value | p-value |
| IL-6 | Intercept | 1 | 0.051166 | 19.54434 | 2.43E-25 |
| | CM | -0.086 | 0.085617 | -1.00448 | 0.319891 |
| | EPA | -0.29014 | 0.072359 | -4.00975 | 0.000199 |
| | CAP | -0.36603 | 0.072359 | -5.05848 | 5.83E-06 |
| | CM:EPA | -0.1487 | 0.117792 | -1.26236 | 0.212561 |
| | CM:CAP | -0.06153 | 0.117792 | -0.52235 | 0.603685 |
| | EPA:CAP | 0.194159 | 0.102331 | 1.897357 | 0.063449 |
| | CM:EPA:CAP | 0.144999 | 0.164209 | 0.883015 | 0.381371 |

Multiple R-squared: 0.662343; F-statistic: 14.29158 on 7 and 51 degrees of freedom

| Table 12: Exploratory factorial regression analyses to examine CAP and EPA interactions of mRNA markers associated with MCF-7 cell growth and inflammation | | | | | |
|---|---|---|---|---|---|
| Marker | IVs | Betas | SE | T-value | p-value |
| IL-8 | Intercept | 1 | 0.060337 | 16.57348 | 3.53E-22 |
| | CM | 0.972652 | 0.095402 | 10.19533 | 6.64E-14 |
| | EPA | -0.33343 | 0.087956 | -3.79089 | 0.000398 |
| | CAP | -0.22486 | 0.08533 | -2.63521 | 0.01111 |
| | CM:EPA | -0.93278 | 0.136594 | -6.82881 | 1.01E-08 |
| | CM:CAP | -0.93251 | 0.134918 | -6.91166 | 7.51E-09 |
| | EPA:CAP | 0.125777 | 0.122546 | 1.026367 | 0.309562 |
| | **CM:EPA:CAP** | **0.897947** | **0.191992** | **4.676997** | **2.17E-05** |

Multiple R-squared: 0.848884; F-statistic: 40.9269 on 7 and 51 degrees of freedom

# Supplementary tables and experimental design for EPA and CAP interactions in adipocytes and breast cancer cell crosstalk

| Table 13: Exploratory factorial regression analyses to examine CAP and EPA interactions of protein markers associated with MCF-7 cell inflammation | | | | | |
|---|---|---|---|---|---|
| Marker | IVs | Betas | SE | T-value | p-value |
| IL-6 | Intercept | 1 | 0.0515 | 19.417 | 5.14E-08 |
| | EPA | -0.10153 | 0.07284 | -1.394 | 2.01E-01 |
| | CAP | -0.11597 | 0.07284 | -1.592 | 1.50E-01 |
| | EPA:CAP | -0.10814 | 0.103 | -1.05 | 3.24E-01 |

Multiple R-squared: 0.7254; F-statistic: 7.043 on 3 and 8 degrees of freedom

| Table 14: Exploratory factorial regression analyses to examine CAP and EPA interactions of protein markers associated with MCF-7 cell inflammation | | | | | |
|---|---|---|---|---|---|
| Marker | IVs | Betas | SE | T-value | p-value |
| IL-8 | Intercept | 1 | 0.06398 | 15.63 | 2.80E-07 |
| | EPA | -0.14349 | 0.09048 | -1.586 | 1.51E-01 |
| | CAP | -0.16202 | 0.09048 | -1.791 | 1.11E-01 |
| | EPA:CAP | 0.22748 | 0.12796 | 1.778 | 1.13E-01 |

Multiple R-squared: 0.3303; F-statistic: 1.315 on 3 and 8 degrees of freedom

| Table 15: Exploratory time-series regression analyses to examine CAP and EPA interactions in MDA-MB-231 cell migration | | | | |
|---|---|---|---|---|
| | Estimate | Std. Error | t value | Pr(>\|t\|) |
| (Intercept) | 66.74926 | 5.4224 | 12.31 | < 2e-16 |
| as.factor(Group)2 | 23.4281 | 7.66843 | 3.055 | 0.0031 |
| as.factor(Group)3 | 2.30795 | 7.66843 | 0.301 | 0.76426 |
| as.factor(Group)4 | 24.12216 | 7.66843 | 3.146 | 0.00237 |
| Time | -1.65706 | 0.21628 | -7.662 | 4.88E-11 |
| as.factor(Group)2:Time | -0.26607 | 0.30586 | -0.87 | 0.3871 |
| as.factor(Group)3:Time | -0.06846 | 0.30586 | -0.224 | 0.82349 |
| as.factor(Group)4:Time | -0.18902 | 0.30586 | -0.618 | 0.53844 |

Residual standard error: 16.38 on 76 degrees of freedom;

Multiple R-squared: 0.7993, Adjusted R-squared: 0.7808;

F-statistic: 43.24 on 7 and 76 DF, p-value: < 2.2e-16;

Group1 (reference) = Control, Group2 = EPA, Group3 = CAP, Group4 = Both

**Supplementary tables and experimental design for EPA and CAP interactions in adipocytes and breast cancer cell crosstalk**

Table 16: Exploratory time-series regression analyses to examine CAP and EPA interactions in MDA-MB-231 cell migration

|  | Estimate | Std. Error | t value | Pr(>|t|) |
|---|---|---|---|---|
| (Intercept) | 92.25178 | 2.01307 | 45.826 | < 2e-16 |
| as.factor(Group)2 | 10.96192 | 2.84691 | 3.85 | 0.000244 |
| as.factor(Group)3 | 13.42635 | 2.84691 | 4.716 | 1.07E-05 |
| as.factor(Group)4 | 12.44705 | 2.84691 | 4.372 | 3.85E-05 |
| Time | -2.13325 | 0.08029 | -26.568 | < 2e-16 |
| as.factor(Group)2:Time | 0.95701 | 0.11355 | 8.428 | 1.67E-12 |
| as.factor(Group)3:Time | 0.81119 | 0.11355 | 7.144 | 4.70E-10 |
| as.factor(Group)4:Time | 0.77482 | 0.11355 | 6.824 | 1.88E-09 |

Residual standard error: 6.079 on 76 degrees of freedom;

Multiple R-squared: 0.9599, Adjusted R-squared: 0.9562;

F-statistic: 259.9 on 7 and 76 DF, p-value: < 2.2e-16;

Group1 (reference) = Control, Group2 = EPA, Group3 = CAP, Group4 = Both

Table 17: Exploratory time-series factorial regression analyses to examine CAP and EPA interactions in MDA-MB-231 cell migration

|  | Estimate | std. Error | t value | Pr(>|t|) |
|---|---|---|---|---|
| (Intercept) | 66.74926 | 5.4224 | 12.31 | < 2e-16 |
| EPA | 23.4281 | 7.66843 | 3.055 | 0.0031 |
| CAP | 2.30795 | 7.66843 | 0.301 | 0.7643 |
| Time | -1.65706 | 0.21628 | -7.662 | 4.88E-11 |
| EPA:CAP | -1.61389 | 10.84479 | -0.149 | 0.8821 |
| EPA:Time | -0.26607 | 0.30586 | -0.87 | 0.3871 |
| CAP:Time | -0.06846 | 0.30586 | -0.224 | 0.8235 |
| EPA:CAP:Time | 0.14552 | 0.43256 | 0.336 | 0.7375 |

Residual standard error: 16.38 on 76 degrees of freedom;

Multiple R-squared: 0.7993, Adjusted R-squared: 0.7808;

F-statistic: 43.24 on 7 and 76 DF, p-value: < 2.2e-16

# Supplementary tables and experimental design for EPA and CAP interactions in adipocytes and breast cancer cell crosstalk

| Table 18: Exploratory time-series factorial regression analyses to examine CAP and EPA interactions in MDA-MB-231 cell migration | | | | |
|---|---|---|---|---|
| | Estimate | std. Error | t value | Pr(>\|t\|) |
| (Intercept) | 92.25178 | 2.01307 | 45.826 | < 2e-16 |
| EPA | 10.96192 | 2.84691 | 3.85 | 0.000244 |
| CAP | 13.42635 | 2.84691 | 4.716 | 1.07E-05 |
| Time | -2.13325 | 0.08029 | -26.568 | < 2e-16 |
| EPA:CAP | -11.9412 | 4.02614 | -2.966 | 0.004031 |
| EPA:Time | 0.95701 | 0.11355 | 8.428 | 1.67E-12 |
| CAP:Time | 0.81119 | 0.11355 | 7.144 | 4.70E-10 |
| EPA:CAP:Time | -0.99338 | 0.16059 | -6.186 | 2.87E-08 |

Residual standard error: 6.079 on 76 degrees of freedom;
Multiple R-squared: 0.9599, Adjusted R-squared: 0.9562;
F-statistic: 259.9 on 7 and 76 DF, p-value: < 2.2e-16
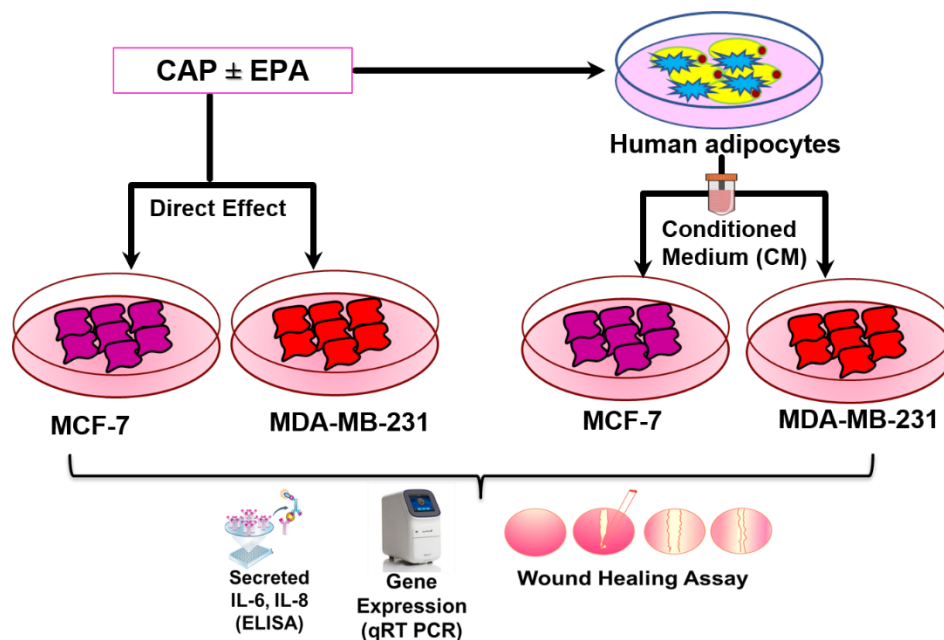


**Figure S1. Experimental design**. MCF-7 and MDA-MB-231 breast cancer cells were treated directly with 100 µM captopril (CAP) with and without (±) 100 µM eicosapentaenoic acid (EPA) Figure 48. hours to investigate the direct effect of CAP and EPA combinations in breast cancer. Next differentiated human adipocytes were treated with 100 µM CAP ± 100 µM EPA followed by collection conditioned medium (CM) after 24 hours. Then breast cancer cells were treated with conditioned medium to investigate the CM mediated effect of adipose RAS inhibition in breast cancer. Gene and protein analyses via qRT PCR and ELISA, and wound healing assays were performed followed by harvesting cells and medium.