

# **Effect of long-term caloric restriction on DNA methylation measures of biological aging in healthy adults from the CALERIE trial**

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## SUPPLEMENTARY TABLES

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**Table S1. DNA-methylation clock and Pace of Aging values for CALERIE™ participants in the Ad Libitum (AL) and Caloric Restriction (CR) groups at baseline, 12-month, and 24-month assessments.** Panel A shows values for original and PC-based versions<sup>18</sup> of first-generation clocks. Panel B shows values for original and PC-based versions of second-generation clocks. Panel C shows values for DunedinPACE. Values for DNA methylation clocks are age-difference scores computed by subtracting participants' chronological ages from their clock ages. DunedinPACE values are not adjusted for chronological age. 95% Confidence intervals are reported in brackets. Follow-up DNA methylation data for the n=69 AL and n=128 CR participants in the analysis sample were available for n=65 AL and n=125 CR participants at 12-months and n=68 AL and 117 CR participants at 24-months. The positive offset observed for the DNAm clocks is a common feature of observations made using EPIC arrays<sup>27</sup>.

#### Panel A. First-Generation Clocks

|                       |          | Ad Libitum (n=69) |                 | Calorie Restriction (n=128) |                 |
|-----------------------|----------|-------------------|-----------------|-----------------------------|-----------------|
|                       |          | Mean              | 95% CI          | Mean                        | 95% CI          |
| Horvath Clock         | Baseline | -2.08             | [-2.94 , -1.22] | -2.06                       | [-2.75 , -1.38] |
|                       | 12mo     | -2.44             | [-3.37 , -1.51] | -2.09                       | [-2.79 , -1.39] |
|                       | 24mo     | -2.38             | [-3.28 , -1.48] | -2.16                       | [-2.89 , -1.43] |
| PC                    | Baseline | 9.06              | [8.07 , 10.04]  | 9.20                        | [8.50 , 9.90]   |
| Horvath Clock         | 12mo     | 8.71              | [7.66 , 9.77]   | 9.02                        | [8.34 , 9.71]   |
|                       | 24mo     | 8.58              | [7.55 , 9.61]   | 8.74                        | [8.00 , 9.49]   |
| Hannum Clock          | Baseline | 3.87              | [3.22 , 4.52]   | 4.17                        | [3.67 , 4.67]   |
|                       | 12mo     | 3.59              | [2.81 , 4.36]   | 3.98                        | [3.45 , 4.50]   |
|                       | 24mo     | 3.75              | [3.05 , 4.46]   | 3.98                        | [3.43 , 4.52]   |
| PC                    | Baseline | 13.44             | [12.58 , 14.29] | 13.64                       | [13.04 , 14.24] |
| Hannum Clock          | 12mo     | 13.24             | [12.28 , 14.19] | 13.46                       | [12.86 , 14.06] |
|                       | 24mo     | 13.11             | [12.22 , 14.00] | 13.29                       | [12.64 , 13.94] |
| Skin & Blood Clock    | Baseline | 1.39              | [0.67 , 2.12]   | 1.60                        | [1.13 , 2.06]   |
|                       | 12mo     | 1.73              | [1.03 , 2.44]   | 1.69                        | [1.24 , 2.15]   |
|                       | 24mo     | 1.92              | [1.16 , 2.69]   | 1.68                        | [1.17 , 2.19]   |
| PC Skin & Blood Clock | Baseline | 6.44              | [5.34 , 7.53]   | 6.92                        | [6.16 , 7.69]   |
|                       | 12mo     | 6.00              | [4.80 , 7.19]   | 6.69                        | [5.95 , 7.44]   |
|                       | 24mo     | 5.71              | [4.57 , 6.85]   | 6.31                        | [5.50 , 7.12]   |

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**Table S1 Continued**

| <b>Panel B. Second-Generation Clocks</b> |          |                          |                  |                                    |                  |
|--|----------|--------------------------|------------------|------------------------------------|------------------|
|  |          | <b>Ad Libitum (n=69)</b> |                  | <b>Calorie Restriction (n=128)</b> |                  |
|  |          | <b>Mean</b>              | <b>95% CI</b>    | <b>Mean</b>                        | <b>95% CI</b>    |
| PhenoAge<br>Clock                        | Baseline | -10.39                   | [-11.53 , -9.24] | -10.14                             | [-11.03 , -9.24] |
|  | 12mo     | -10.59                   | [-11.82 , -9.35] | -10.10                             | [-11.04 , -9.16] |
|  | 24mo     | -10.99                   | [-12.12 , -9.86] | -10.12                             | [-11.00 , -9.24] |
| PC                                       | Baseline | 2.48                     | [1.36 , 3.61]    | 2.66                               | [1.81 , 3.51]    |
| PhenoAge                                 | 12mo     | 2.40                     | [1.24 , 3.55]    | 2.36                               | [1.53 , 3.19]    |
| Clock                                    | 24mo     | 2.23                     | [1.12 , 3.35]    | 2.51                               | [1.67 , 3.36]    |
|  |          |                          |                  |                                    |                  |
| GrimAge<br>Clock                         | Baseline | 4.85                     | [4.14 , 5.56]    | 4.78                               | [4.32 , 5.24]    |
|  | 12mo     | 4.63                     | [3.93 , 5.33]    | 4.33                               | [3.88 , 4.78]    |
|  | 24mo     | 4.25                     | [3.61 , 4.90]    | 4.06                               | [3.59 , 4.54]    |
| PC                                       | Baseline | 11.51                    | [10.88 , 12.14]  | 11.85                              | [11.34 , 12.35]  |
| GrimAge                                  | 12mo     | 11.23                    | [10.53 , 11.93]  | 11.42                              | [10.92 , 11.92]  |
| Clock                                    | 24mo     | 10.79                    | [10.16 , 11.43]  | 11.08                              | [10.56 , 11.60]  |
|  |          |                          |                  |                                    |                  |
| <b>Panel C. Pace of Aging</b>            |          |                          |                  |                                    |                  |
| Dunedin                                  | Baseline | 0.94                     | [0.92 , 0.96]    | 0.95                               | [0.93 , 0.96]    |
| PACE                                     | 12mo     | 0.95                     | [0.93 , 0.97]    | 0.93                               | [0.92 , 0.94]    |
|  | 24mo     | 0.95                     | [0.93 , 0.97]    | 0.93                               | [0.92 , 0.95]    |

Table entries for measurements included in primary analysis (PC PhenoAge, PC GrimAge, DunedinPACE) are highlighted in black text.

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**Table S2. Technical reliability and within-individual stability of DNAm clocks and DunedinPACE in GSE55763 and the CALERIE™ trial.** Panel A shows values for original and PC-based versions<sup>18</sup> of first-generation clocks. Panel B shows values for original and PC-based versions of second-generation clocks. Panel C shows values for DunedinPACE. The left side of the table reports technical (test-retest) reliability across technical replicates from n=36 samples measured in duplicate in GSE55763. The right side of the table reports within-individual stability across repeated measurements at baseline, 12months, and 24 months in the CALERIE™ trial. Reliabilities are calculated as intra-class correlation coefficients (ICCs). Pearson r correlation are also reported.

| Technical Reliability (GSE55763)  |       |               |         | Within-Individual Stability (CALERIE™) |      |                |      |      |               |
|-----------------------------------|-------|---------------|---------|--|------|----------------|------|------|---------------|
|                                   |       |               |         |  | AL   |                | CR   |      |               |
|                                   | ICC   | 95% CI        |         | r                                      | ICC  | 95% CI         | r    | ICC  | 95% CI        |
| Panel A. First-Generation Clocks  |       |               |         |  |      |                |      |      |               |
| Horvath Clock                     | 0.824 | [0.68 , 0.97] | BI-12mo | 0.79                                   | 0.78 | [-0.62 , 2.29] | 0.78 | 0.79 | [0.71 , 0.85] |
|                                   |       |               | BI-24mo | 0.74                                   | 0.71 | [0.57 , 0.81]  | 0.82 | 0.83 | [0.77 , 0.88] |
| PC Horvath Clock                  | 0.969 | [0.95 , 0.99] | BI-12mo | 0.97                                   | 0.97 | [0.95 , 0.98]  | 0.96 | 0.96 | [0.94 , 0.97] |
|                                   |       |               | BI-24mo | 0.97                                   | 0.96 | [0.93 , 0.97]  | 0.97 | 0.96 | [0.94 , 0.97] |
|                                   |       |               |         |  |      |                |      |      |               |
| Hannum Clock                      | 0.853 | [0.74 , 0.96] | BI-12mo | 0.84                                   | 0.83 | [0.74 , 0.89]  | 0.79 | 0.78 | [0.71 , 0.84] |
|                                   |       |               | BI-24mo | 0.82                                   | 0.82 | [0.72 , 0.88]  | 0.80 | 0.79 | [0.72 , 0.85] |
| PC Hannum Clock                   | 0.986 | [0.98 , 0.99] | BI-12mo | 0.95                                   | 0.95 | [0.91 , 0.97]  | 0.91 | 0.91 | [0.88 , 0.94] |
|                                   |       |               | BI-24mo | 0.92                                   | 0.92 | [0.87 , 0.95]  | 0.93 | 0.93 | [0.90 , 0.95] |
|                                   |       |               |         |  |      |                |      |      |               |
| Skin & Blood Clock                | 0.882 | [0.75 , 1.01] | BI-12mo | 0.83                                   | 0.83 | [0.73 , 0.89]  | 0.76 | 0.76 | [0.68 , 0.83] |
|                                   |       |               | BI-24mo | 0.82                                   | 0.81 | [0.71 , 0.88]  | 0.85 | 0.85 | [0.79 , 0.89] |
| PC Skin & Blood Clock             | 0.978 | [0.96 , 0.99] | BI-12mo | 0.96                                   | 0.96 | [0.93 , 0.97]  | 0.95 | 0.95 | [0.93 , 0.96] |
|                                   |       |               | BI-24mo | 0.95                                   | 0.94 | [0.90 , 0.96]  | 0.96 | 0.95 | [0.93 , 0.97] |
| Panel B. Second-Generation Clocks |       |               |         |  |      |                |      |      |               |
| PhenoAge Clock                    | 0.727 | [0.55 , 0.91] | BI-12mo | 0.74                                   | 0.75 | [0.62 , 0.84]  | 0.74 | 0.74 | [0.65 , 0.81] |
|                                   |       |               | BI-24mo | 0.62                                   | 0.62 | [0.45 , 0.75]  | 0.81 | 0.81 | [0.74 , 0.87] |
| PC PhenoAge Clock                 | 0.987 | [0.98 , 1.00] | BI-12mo | 0.85                                   | 0.85 | [0.77 , 0.91]  | 0.82 | 0.82 | [0.76 , 0.87] |
|                                   |       |               | BI-24mo | 0.79                                   | 0.80 | [0.69 , 0.87]  | 0.89 | 0.89 | [0.84 , 0.92] |
|                                   |       |               |         |  |      |                |      |      |               |
| GrimAge Clock                     | 0.96  | [0.93 , 0.99] | BI-12mo | 0.91                                   | 0.92 | [0.87 , 0.95]  | 0.83 | 0.80 | [0.72 , 0.85] |
|                                   |       |               | BI-24mo | 0.87                                   | 0.85 | [0.77 , 0.91]  | 0.86 | 0.80 | [0.72 , 0.86] |
| PC GrimAge Clock                  | 0.988 | [0.97 , 1.00] | BI-12mo | 0.93                                   | 0.91 | [0.86 , 0.95]  | 0.91 | 0.86 | [0.81 , 0.90] |
|                                   |       |               | BI-24mo | 0.87                                   | 0.83 | [0.74 , 0.89]  | 0.93 | 0.88 | [0.83 , 0.92] |
| Panel C. Pace of Aging            |       |               |         |  |      |                |      |      |               |
| DunedinPACE                       | 0.964 | [0.93 , 1.00] | BI-12mo | 0.85                                   | 0.84 | [0.75 , 0.90]  | 0.77 | 0.75 | [0.67 , 0.82] |
|                                   |       |               | BI-24mo | 0.84                                   | 0.83 | [0.74 , 0.89]  | 0.80 | 0.79 | [0.71 , 0.85] |

Table entries for measurements included in primary analysis (PC PhenoAge, PC GrimAge, DunedinPACE) are highlighted in black text.

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**Table S3. Change scores for DNA-methylation measures of aging at 12-and 24-month follow-ups in CALERIE™-Trial Ad Libitum and Caloric Restriction group participants.** Panel A shows change scores for original and PC-based versions<sup>18</sup> of first-generation clocks. Panel B shows change scores for original and PC-based versions of second-generation clocks. Panel C shows change scores for DunedinPACE. The table shows change-scores computed relative to baseline for the aging measures at 12- and 24-month follow-ups in the AL and CR groups. Change scores are scaled in units of the aging measures' standard deviations at pre-treatment baseline. Change scores can be back-converted into the original units of the measures by multiplying by the standard deviation. The standard deviations are as follows: Horvath Clock = 3.84; PC Horvath Clock = 4.07; Hannum Clock = 2.85; PC Hannum Clock = 3.50; Skin & Blood Clock = 2.81; PC Skin & Blood Clock = 4.46; PhenoAge Clock = 5.04; PC PhenoAge Clock = 4.84; GrimAge Clock = 2.76; PC GrimAge Clock = 2.82; DunedinPACE = 0.09. For the clocks, change scores are computed from age-difference values (i.e. clock age – chronological age) at baseline and the 12- and 24-month follow-ups.

|  |      | Ad Libitum (n=69) |                 | Calorie Restriction (n=128) |                 |
|--|------|-------------------|-----------------|-----------------------------|-----------------|
|  |      | Mean              | 95% CI          | Mean                        | 95% CI          |
| <b>Panel A. First-Generation Clocks</b>  |      |                   |                 |                             |                 |
| Horvath Clock                            | 12mo | -0.08             | [-0.23 , 0.07]  | -0.02                       | [-0.14 , 0.10]  |
|  | 24mo | -0.10             | [-0.27 , 0.07]  | -0.06                       | [-0.17 , 0.05]  |
| PC Horvath Clock                         | 12mo | -0.08             | [-0.14 , -0.02] | -0.07                       | [-0.12 , -0.02] |
|  | 24mo | -0.15             | [-0.21 , -0.08] | -0.11                       | [-0.15 , -0.06] |
| Hannum Clock                             | 12mo | -0.11             | [-0.25 , 0.04]  | -0.09                       | [-0.21 , 0.03]  |
|  | 24mo | -0.06             | [-0.21 , 0.09]  | -0.06                       | [-0.18 , 0.06]  |
| PC Hannum Clock                          | 12mo | -0.07             | [-0.15 , 0.02]  | -0.07                       | [-0.14 , 0.01]  |
|  | 24mo | -0.13             | [-0.23 , -0.03] | -0.09                       | [-0.16 , -0.02] |
| Skin & Blood Clock                       | 12mo | 0.14              | [-0.01 , 0.29]  | 0.03                        | [-0.08 , 0.14]  |
|  | 24mo | 0.17              | [0.01 , 0.33]   | 0.00                        | [-0.09 , 0.10]  |
| PC Skin & Blood Clock                    | 12mo | -0.10             | [-0.17 , -0.02] | -0.08                       | [-0.14 , -0.03] |
|  | 24mo | -0.19             | [-0.26 , -0.11] | -0.12                       | [-0.17 , -0.07] |
| <b>Panel B. Second-Generation Clocks</b> |      |                   |                 |                             |                 |
| PhenoAge Clock                           | 12mo | 0.00              | [-0.17 , 0.17]  | 0.00                        | [-0.14 , 0.13]  |
|  | 24mo | -0.14             | [-0.34 , 0.06]  | 0.03                        | [-0.09 , 0.14]  |
| PC PhenoAge Clock                        | 12mo | -0.02             | [-0.15 , 0.11]  | -0.06                       | [-0.17 , 0.04]  |
|  | 24mo | -0.08             | [-0.22 , 0.07]  | -0.02                       | [-0.11 , 0.06]  |
| GrimAge Clock                            | 12mo | -0.07             | [-0.18 , 0.04]  | -0.19                       | [-0.28 , -0.09] |
|  | 24mo | -0.23             | [-0.36 , -0.11] | -0.23                       | [-0.33 , -0.14] |
| PC GrimAge Clock                         | 12mo | -0.09             | [-0.19 , 0.00]  | -0.17                       | [-0.24 , -0.09] |
|  | 24mo | -0.26             | [-0.37 , -0.14] | -0.22                       | [-0.29 , -0.15] |
| <b>Panel C. Pace of Aging</b>            |      |                   |                 |                             |                 |
| DunedinPACE                              | 12mo | 0.13              | [-0.01 , 0.27]  | -0.18                       | [-0.29 , -0.06] |
|  | 24mo | 0.12              | [-0.01 , 0.26]  | -0.12                       | [-0.23 , -0.01] |

Table entries for measurements included in primary analysis (PC PhenoAge, PC GrimAge, DunedinPACE) are highlighted in black text.

# SUPPLEMENTARY TABLES

**Table S4. Effect-size estimates for intent-to-treat (ITT) and effect of treatment-on-the-treated (TOT) analysis testing the effect of CALERIE™ intervention on DNA methylation measures of aging.** Panel A shows treatment effects for original and PC-based versions<sup>18</sup> of first-generation clocks. Panel B shows treatment effects for original and PC-based versions of second-generation clocks. Panel C shows treatment effects for DunedinPACE. Intent to treat (ITT) effect estimates are reported on the left side of the table. Effect of treatment-on-the-treated (TOT) effect estimates are reported on the right side of the table. ITT analysis was conducted using mixed models to compare change-scores for DNA methylation measures of aging between CR and AL groups. Models included covariates for sex, race/ethnicity, study site, and pre-treatment baseline chronological age and body-mass index stratum. TOT analysis was conducted using instrumental variables regression to test associations of CR dose with aging-measure change-scores. Instruments included treatment group and interactions between treatment group and sex, body-mass index, and baseline levels of the aging measures. Model covariates were the same as in ITT analysis. TOT effect estimates are reported for a 20% dose of CR. This level of CR represents the 75<sup>th</sup> percentile of the treatment group CR distribution at 12-months follow-up and the 87<sup>th</sup> percentile of the treatment group CR distribution at 24-months follow-up. Effect-sizes are scaled in terms of standard deviations of the measures at pre-intervention baseline so they can be interpreted on a Cohen's d scale. Effect-sizes can be back-converted into the original units of the measures by multiplying the Cohen's d effect-size by the standard deviation. All tests reported were two-sided. The standard deviations are as follows: Horvath Clock = 3.84; PC Horvath Clock = 4.07; Hannum Clock = 2.85; PC Hannum Clock = 3.50; Skin & Blood Clock = 2.81; PC Skin & Blood Clock = 4.46; PhenoAge Clock = 5.04; PC PhenoAge Clock = 4.84; GrimAge Clock = 2.76; PC GrimAge Clock = 2.82; DunedinPACE = 0.09.

**Panel A. First Generation Clocks**

|                       |      | Intent-to-Treat (ITT): Difference between CR and AL |                |         | Effect of Treatment on the Treated (TOT): Estimated Effect of 20% CR |                |         |
|-----------------------|------|---|----------------|---------|--|----------------|---------|
|                       |      | Treatment Effect (Cohen's d)                        | 95% CI         | p-value | Treatment Effect (Cohen's d)   | 95% CI         | p-value |
| Horvath Clock         | 12mo | 0.07  | [-0.11 , 0.25] | 0.457   | 0.14   | [-0.10 , 0.39] | 0.249   |
|                       | 24mo | 0.05  | [-0.14 , 0.24] | 0.599   | 0.10   | [-0.22 , 0.42] | 0.526   |
| PC Horvath Clock      | 12mo | 0.01  | [-0.06 , 0.09] | 0.771   | 0.05   | [-0.06 , 0.15] | 0.375   |
|                       | 24mo | 0.04  | [-0.04 , 0.12] | 0.341   | 0.05   | [-0.08 , 0.19] | 0.438   |
| Hannum Clock          | 12mo | 0.03  | [-0.15 , 0.21] | 0.758   | 0.09   | [-0.15 , 0.33] | 0.484   |
|                       | 24mo | 0.00  | [-0.18 , 0.18] | 0.986   | -0.02  | [-0.35 , 0.31] | 0.909   |
| PC Hannum Clock       | 12mo | 0.00  | [-0.11 , 0.11] | 0.964   | 0.02   | [-0.14 , 0.17] | 0.845   |
|                       | 24mo | 0.04  | [-0.08 , 0.15] | 0.501   | 0.03   | [-0.16 , 0.23] | 0.753   |
| Skin & Blood Clock    | 12mo | -0.09   | [-0.26 , 0.08] | 0.316   | -0.06  | [-0.30 , 0.18] | 0.618   |
|                       | 24mo | -0.14   | [-0.32 , 0.04] | 0.139   | -0.19  | [-0.49 , 0.10] | 0.197   |
| PC Skin & Blood Clock | 12mo | 0.02  | [-0.07 , 0.11] | 0.647   | 0.05   | [-0.07 , 0.17] | 0.390   |
|                       | 24mo | 0.06  | [-0.02 , 0.15] | 0.156   | 0.07   | [-0.07 , 0.22] | 0.314   |

# SUPPLEMENTARY TABLES

## Table S4 Continued

### Panel B. Second Generation Clocks

| Intent-to-Treat (ITT): Difference between<br>CR and AL |      |                       |                |         | Effect of Treatment on the Treated (TOT):<br>Estimated Effect of 20% CR |                |       |
|--|------|-----------------------|----------------|---------|---|----------------|-------|
|  |      | Effect<br>(Cohen's d) | 95% CI         | p-value |   |                |       |
|  |      | Effect<br>(Cohen's d) | 95% CI         | p-value |   |                |       |
| PhenoAge   | 12mo | 0.04                  | [-0.17 , 0.24] | 0.719   | -0.02   | [-0.29 , 0.26] | 0.889 |
| Clock  | 24mo | 0.16                  | [-0.03 , 0.36] | 0.104   | 0.21  | [-0.09 , 0.51] | 0.174 |
| PC   | 12mo | -0.03                 | [-0.19 , 0.12] | 0.663   | -0.08   | [-0.31 , 0.14] | 0.473 |
| PhenoAge   | 24mo | 0.05                  | [-0.11 , 0.20] | 0.550   | 0.00  | [-0.24 , 0.25] | 0.977 |
| GrimAge  | 12mo | -0.10                 | [-0.23 , 0.04] | 0.170   | -0.14   | [-0.34 , 0.06] | 0.164 |
| Clock  | 24mo | -0.01                 | [-0.14 , 0.12] | 0.898   | -0.09   | [-0.32 , 0.14] | 0.425 |
| PC GrimAge   | 12mo | -0.04                 | [-0.16 , 0.07] | 0.461   | -0.13   | [-0.30 , 0.05] | 0.154 |
| Clock  | 24mo | 0.05                  | [-0.07 , 0.17] | 0.432   | 0.01  | [-0.17 , 0.19] | 0.902 |

### Panel C. Pace of Aging

|         |      |              |                        |                 |              |                        |                 |
|---------|------|--------------|------------------------|-----------------|--------------|------------------------|-----------------|
| Dunedin | 12mo | <b>-0.29</b> | <b>[-0.45 , -0.13]</b> | <b>4.83E-04</b> | <b>-0.43</b> | <b>[-0.67 , -0.19]</b> | <b>3.69E-04</b> |
| PACE    | 24mo | <b>-0.25</b> | <b>[-0.41 , -0.09]</b> | <b>0.002</b>    | <b>-0.40</b> | <b>[-0.67 , -0.12]</b> | <b>0.004</b>    |

Table entries for measurements included in primary analysis (PC PhenoAge, PC GrimAge, DunedinPACE) are highlighted in black text. Bolded entries fall below the significance threshold of  $p < 0.005$ .

**Table S5. Analysis of CALERIE™ intervention effects with treatment-group stratified by dose of calorie restriction.** Panel A shows treatment effects for original and PC-based versions<sup>18</sup> of first-generation clocks. Panel B shows treatment effects for original and PC-based versions of second-generation clocks. Panel C shows treatment effects for DunedinPACE. The table reports effect-size estimates from analysis using the same regression model used to estimate ITT effects (**Table S4**) with the treatment group stratified by the percent calorie restriction achieved by participants in the CR-intervention group. We used a threshold value of 10% CR relative to baseline to stratify the treatment group. The <10% CR group included n=36 participants at 12-months follow-up and n=48 participants at 24-months follow-up. The >10% CR group included n=89 participants at 12-months follow-up and n=69 participants at 24-months follow-up. All tests reported were two-sided.

**Panel A. First-Generation Clocks**

|                       |         |      | Treatment Effect |               |         |
|-----------------------|---------|------|------------------|---------------|---------|
|                       |         |      | (Cohen's d)      | 95% CI        | p-value |
| Horvath Clock         | <10% CR | 12mo | 0.13             | [-0.13, 0.38] | 0.331   |
|                       |         | 24mo | 0.07             | [-0.15, 0.29] | 0.542   |
|                       | >10% CR | 12mo | 0.05             | [-0.15, 0.24] | 0.641   |
|                       |         | 24mo | 0.04             | [-0.17, 0.24] | 0.740   |
| PC Horvath Clock      | <10% CR | 12mo | 0.00             | [-0.10, 0.11] | 0.963   |
|                       |         | 24mo | 0.06             | [-0.04, 0.16] | 0.227   |
|                       | >10% CR | 12mo | 0.02             | [-0.06, 0.10] | 0.713   |
|                       |         | 24mo | 0.02             | [-0.06, 0.10] | 0.599   |
| Hannum Clock          | <10% CR | 12mo | -0.01            | [-0.25, 0.23] | 0.945   |
|                       |         | 24mo | -0.02            | [-0.22, 0.18] | 0.857   |
|                       | >10% CR | 12mo | 0.04             | [-0.15, 0.24] | 0.660   |
|                       |         | 24mo | 0.02             | [-0.19, 0.22] | 0.874   |
| PC Hannum Clock       | <10% CR | 12mo | -0.01            | [-0.17, 0.16] | 0.942   |
|                       |         | 24mo | 0.07             | [-0.08, 0.22] | 0.347   |
|                       | >10% CR | 12mo | 0.01             | [-0.11, 0.12] | 0.914   |
|                       |         | 24mo | 0.02             | [-0.10, 0.14] | 0.767   |
| Skin & Blood Clock    | <10% CR | 12mo | -0.09            | [-0.30, 0.13] | 0.428   |
|                       |         | 24mo | -0.18            | [-0.39, 0.03] | 0.095   |
|                       | >10% CR | 12mo | -0.09            | [-0.28, 0.10] | 0.350   |
|                       |         | 24mo | -0.11            | [-0.31, 0.09] | 0.283   |
| PC Skin & Blood Clock | <10% CR | 12mo | 0.00             | [-0.12, 0.12] | 1.000   |
|                       |         | 24mo | 0.09             | [-0.02, 0.20] | 0.105   |
|                       | >10% CR | 12mo | 0.03             | [-0.06, 0.12] | 0.527   |
|                       |         | 24mo | 0.05             | [-0.04, 0.14] | 0.316   |



Table S5 Continued

| Panel B. Second-Generation Clocks |         |      |                                 |                 |          |
|-----------------------------------|---------|------|---------------------------------|-----------------|----------|
|                                   |         |      | Treatment Effect<br>(Cohen's d) | 95% CI          | p-value  |
| PhenoAge Clock                    | <10% CR | 12mo | 0.17                            | [-0.10 , 0.44]  | 0.219    |
|                                   |         | 24mo | 0.10                            | [-0.13 , 0.34]  | 0.392    |
|                                   | >10% CR | 12mo | -0.02                           | [-0.24 , 0.21]  | 0.888    |
|                                   |         | 24mo | 0.20                            | [-0.01 , 0.41]  | 0.062    |
| PC PhenoAge Clock                 | <10% CR | 12mo | -0.07                           | [-0.30 , 0.16]  | 0.540    |
|                                   |         | 24mo | 0.05                            | [-0.13 , 0.24]  | 0.580    |
|                                   | >10% CR | 12mo | -0.02                           | [-0.19 , 0.14]  | 0.811    |
|                                   |         | 24mo | 0.04                            | [-0.12 , 0.21]  | 0.591    |
| GrimAge Clock                     | <10% CR | 12mo | -0.12                           | [-0.29 , 0.05]  | 0.167    |
|                                   |         | 24mo | -0.07                           | [-0.24 , 0.09]  | 0.383    |
|                                   | >10% CR | 12mo | -0.09                           | [-0.24 , 0.07]  | 0.266    |
|                                   |         | 24mo | 0.04                            | [-0.10 , 0.18]  | 0.598    |
| PC GrimAge Clock                  | <10% CR | 12mo | -0.06                           | [-0.22 , 0.10]  | 0.492    |
|                                   |         | 24mo | 0.05                            | [-0.09 , 0.19]  | 0.454    |
|                                   | >10% CR | 12mo | -0.04                           | [-0.17 , 0.09]  | 0.539    |
|                                   |         | 24mo | 0.04                            | [-0.08 , 0.17]  | 0.496    |
| Panel C. Pace of Aging            |         |      |                                 |                 |          |
| Dunedin                           | <10% CR | 12mo | -0.19                           | [-0.41 , 0.04]  | 0.099    |
|                                   |         | 24mo | -0.14                           | [-0.34 , 0.06]  | 0.166    |
| PACE                              | >10% CR | 12mo | -0.33                           | [-0.51 , -0.15] | 2.31E-04 |
|                                   |         | 24mo | -0.33                           | [-0.50 , -0.15] | 2.63E-04 |

Table entries for measurements included in primary analysis (PC PhenoAge, PC GrimAge, DunedinPACE) are highlighted in black text. Bolded entries fall below the significance threshold of  $p < 0.005$ .

**Table S6. Cell-count-adjusted effect-size estimates for intent-to-treat (ITT) and effect of treatment-on-the-treated (TOT) analysis testing the effect of CALERIE™ intervention on DNA methylation measures of aging.** Panel A shows treatment effects for original and PC-based versions<sup>18</sup> of first-generation clocks. Panel B shows treatment effects for original and PC-based versions of second-generation clocks. Panel C shows treatment effects for DunedinPACE. Intent to treat (ITT) effect estimates are reported on the left side of the table. Effect of treatment-on-the-treated (TOT) effect estimates are reported on the right side of the table. Models are the same as those reported in **Table S4**, with addition of covariates for change scores of estimated counts of monocytes, lymphocytes (CD4, CD8, natural killer, and b-cells), and neutrophils computed using the R package FlowSorted.Blood.EPIC. All tests reported were two-sided.

| Intent-to-Treat (ITT): Difference between CR and AL |      |       |                 |          | Effect of Treatment on the Treated (TOT):<br>Estimated Effect of 20% CR |  |       |
|---|------|-------|-----------------|----------|---|--|-------|
| Treatment Effect<br>(Cohen's d)                     |      |       |                 |          | Treatment Effect<br>(Cohen's d)   |  |       |
| 95% CI  |      |       |                 |          | 95% CI  |  |       |
| p-value   |      |       |                 |          | p-value   |  |       |
| Panel A. First-Generation Clocks                    |      |       |                 |          |   |  |       |
| Horvath Clock                                       | 12mo | 0.11  | [-0.07 , 0.29]  | 0.230    | 0.16 [-0.09 , 0.42]   |  | 0.198 |
|   | 24mo | 0.08  | [-0.11 , 0.27]  | 0.429    | 0.15 [-0.17 , 0.47]   |  | 0.356 |
| PC Horvath Clock                                    | 12mo | 0.04  | [-0.02 , 0.10]  | 0.197    | 0.07 [-0.02 , 0.15]   |  | 0.123 |
|   | 24mo | 0.03  | [-0.03 , 0.09]  | 0.333    | 0.08 [-0.03 , 0.19]   |  | 0.156 |
| Hannum Clock  | 12mo | 0.06  | [-0.12 , 0.23]  | 0.513    | 0.09 [-0.15 , 0.34]   |  | 0.454 |
|   | 24mo | -0.01 | [-0.18 , 0.17]  | 0.947    | -0.05 [-0.36 , 0.25]  |  | 0.725 |
| PC Hannum Clock                                     | 12mo | 0.06  | [-0.01 , 0.14]  | 0.097    | 0.09 [-0.02 , 0.19]   |  | 0.097 |
|   | 24mo | 0.05  | [-0.04 , 0.14]  | 0.269    | 0.09 [-0.06 , 0.23]   |  | 0.236 |
| Skin & Blood Clock                                  | 12mo | -0.09 | [-0.26 , 0.09]  | 0.322    | -0.10 [-0.34 , 0.13]  |  | 0.384 |
|   | 24mo | -0.12 | [-0.31 , 0.06]  | 0.187    | -0.20 [-0.50 , 0.10]  |  | 0.191 |
| PC Skin & Blood Clock                               | 12mo | 0.04  | [-0.02 , 0.11]  | 0.206    | 0.07 [-0.02 , 0.16]   |  | 0.111 |
|   | 24mo | 0.04  | [-0.03 , 0.11]  | 0.263    | 0.08 [-0.03 , 0.19]   |  | 0.138 |
| Panel B. Second-Generation Clocks                   |      |       |                 |          |   |  |       |
| PhenoAge Clock                                      | 12mo | 0.04  | [-0.14 , 0.22]  | 0.664    | 0.05 [-0.21 , 0.30]   |  | 0.724 |
|   | 24mo | 0.10  | [-0.08 , 0.27]  | 0.280    | 0.14 [-0.13 , 0.42]   |  | 0.308 |
| PC PhenoAge   | 12mo | 0.01  | [-0.06 , 0.08]  | 0.746    | 0.02 [-0.08 , 0.11]   |  | 0.685 |
|   | 24mo | 0.01  | [-0.07 , 0.09]  | 0.846    | 0.03 [-0.10 , 0.15]   |  | 0.692 |
| GrimAge Clock                                       | 12mo | -0.08 | [-0.20 , 0.04]  | 0.213    | -0.10 [-0.28 , 0.07]  |  | 0.252 |
|   | 24mo | -0.05 | [-0.18 , 0.08]  | 0.470    | -0.10 [-0.32 , 0.13]  |  | 0.396 |
| PC GrimAge Clock                                    | 12mo | -0.03 | [-0.08 , 0.02]  | 0.248    | -0.05 [-0.12 , 0.03]  |  | 0.210 |
|   | 24mo | 0.00  | [-0.06 , 0.05]  | 0.884    | -0.03 [-0.12 , 0.06]  |  | 0.544 |
| Panel C. Pace of Aging                              |      |       |                 |          |   |  |       |
| Dunedin PACE  | 12mo | -0.26 | [-0.42 , -0.10] | 1.29E-03 | -0.36 [-0.59 , -0.13]   |  | 0.002 |
| PACE  | 24mo | -0.24 | [-0.40 , -0.07] | 0.005    | -0.30 [-0.58 , -0.02]   |  | 0.033 |

Table entries for measurements included in primary analysis (PC PhenoAge, PC GrimAge, DunedinPACE) are highlighted in black text. Bolded entries fall below the significance threshold of  $p < 0.005$ .

**Table S7 Means for DNA-methylation measures of aging over follow-up for women and men in the CALERIE™ Trial.** Data are the same as reported in **Table S1** disaggregated by sex. Panel A shows values for original and PC-based versions<sup>18</sup> of first-generation clocks. Panel B shows values for original and PC-based versions of second-generation clocks. Panel C shows values for DunedinPACE.

**Panel A. First-Generation Clocks**

|                       |          | Women             |                |                            |                | Men               |                |                            |                |
|-----------------------|----------|-------------------|----------------|----------------------------|----------------|-------------------|----------------|----------------------------|----------------|
|                       |          | Ad Libitum (n=48) |                | Calorie Restriction (n=89) |                | Ad Libitum (n=21) |                | Calorie Restriction (n=39) |                |
|                       |          | Mean              | 95% CI         | Mean                       | 95% CI         | Mean              | 95% CI         | Mean                       | 95% CI         |
| Horvath Clock         | Baseline | -2.36             | [-3.30, -1.43] | -2.44                      | [-3.30, -1.58] | -1.43             | [-3.30, 0.45]  | -1.21                      | [-2.29, -0.12] |
|                       | 12mo     | -2.39             | [-3.37, -1.41] | -2.45                      | [-3.34, -1.57] | -2.54             | [-4.68, -0.40] | -1.22                      | [-2.29, -0.15] |
|                       | 24mo     | -2.40             | [-3.39, -1.41] | -2.59                      | [-3.51, -1.68] | -2.34             | [-4.29, -0.40] | -1.23                      | [-2.35, -0.10] |
| PC Horvath Clock      | Baseline | 8.74              | [7.62, 9.87]   | 8.83                       | [8.02, 9.63]   | 9.77              | [7.77, 11.77]  | 10.04                      | [8.68, 11.40]  |
|                       | 12mo     | 8.50              | [7.25, 9.75]   | 8.66                       | [7.86, 9.47]   | 9.20              | [7.18, 11.21]  | 9.88                       | [8.57, 11.19]  |
|                       | 24mo     | 8.25              | [7.04, 9.45]   | 8.53                       | [7.66, 9.41]   | 9.33              | [7.36, 11.31]  | 9.20                       | [7.76, 10.63]  |
| Hannum Clock          | Baseline | 3.54              | [2.71, 4.37]   | 4.19                       | [3.61, 4.76]   | 4.62              | [3.71, 5.54]   | 4.13                       | [3.10, 5.16]   |
|                       | 12mo     | 3.41              | [2.39, 4.43]   | 3.96                       | [3.32, 4.59]   | 3.98              | [2.95, 5.02]   | 4.02                       | [3.08, 4.97]   |
|                       | 24mo     | 3.54              | [2.71, 4.37]   | 4.06                       | [3.40, 4.71]   | 4.23              | [2.90, 5.57]   | 3.80                       | [2.80, 4.81]   |
| PC Hannum Clock       | Baseline | 12.94             | [11.95, 13.93] | 13.34                      | [12.63, 14.05] | 14.57             | [12.96, 16.18] | 14.32                      | [13.21, 15.44] |
|                       | 12mo     | 12.79             | [11.64, 13.94] | 13.19                      | [12.47, 13.92] | 14.27             | [12.57, 15.96] | 14.09                      | [13.05, 15.14] |
|                       | 24mo     | 12.53             | [11.49, 13.57] | 13.17                      | [12.37, 13.97] | 14.41             | [12.81, 16.01] | 13.55                      | [12.43, 14.68] |
| Skin & Blood Clock    | Baseline | 1.60              | [0.83, 2.37]   | 1.60                       | [0.99, 2.21]   | 0.92              | [-0.73, 2.57]  | 1.58                       | [0.94, 2.23]   |
|                       | 12mo     | 2.02              | [1.21, 2.83]   | 1.74                       | [1.18, 2.30]   | 1.06              | [-0.32, 2.45]  | 1.58                       | [0.80, 2.36]   |
|                       | 24mo     | 2.29              | [1.47, 3.11]   | 1.70                       | [1.01, 2.38]   | 1.10              | [-0.54, 2.74]  | 1.66                       | [1.01, 2.30]   |
| PC Skin & Blood Clock | Baseline | 6.44              | [5.10, 7.78]   | 6.90                       | [6.02, 7.79]   | 6.43              | [4.50, 8.36]   | 6.97                       | [5.47, 8.46]   |
|                       | 12mo     | 6.04              | [4.55, 7.53]   | 6.64                       | [5.77, 7.52]   | 5.90              | [3.83, 7.96]   | 6.82                       | [5.38, 8.25]   |
|                       | 24mo     | 5.61              | [4.19, 7.04]   | 6.44                       | [5.50, 7.39]   | 5.92              | [3.98, 7.86]   | 6.01                       | [4.44, 7.58]   |

**Panel B. Second-Generation Clocks**

|                   |          | Women             |                 |                            |                 | Men               |                  |                            |                  |
|-------------------|----------|-------------------|-----------------|----------------------------|-----------------|-------------------|------------------|----------------------------|------------------|
|                   |          | Ad Libitum (n=48) |                 | Calorie Restriction (n=89) |                 | Ad Libitum (n=21) |                  | Calorie Restriction (n=39) |                  |
|                   |          | Mean              | 95% CI          | Mean                       | 95% CI          | Mean              | 95% CI           | Mean                       | 95% CI           |
| PhenoAge Clock    | Baseline | -9.56             | [-10.88, -8.23] | -9.50                      | [-10.58, -8.43] | -12.28            | [-14.34, -10.22] | -11.59                     | [-13.15, -10.04] |
|                   | 12mo     | -10.10            | [-11.63, -8.57] | -9.34                      | [-10.41, -8.28] | -11.70            | [-13.73, -9.67]  | -11.91                     | [-13.73, -10.10] |
|                   | 24mo     | -10.33            | [-11.73, -8.93] | -9.38                      | [-10.39, -8.37] | -12.47            | [-14.23, -10.71] | -11.71                     | [-13.35, -10.08] |
| PC PhenoAge Clock | Baseline | 2.90              | [1.64, 4.15]    | 2.96                       | [1.94, 3.99]    | 1.53              | [-0.81, 3.87]    | 1.98                       | [0.44, 3.52]     |
|                   | 12mo     | 2.83              | [1.49, 4.16]    | 2.63                       | [1.67, 3.58]    | 1.41              | [-0.87, 3.69]    | 1.73                       | [0.06, 3.41]     |
|                   | 24mo     | 2.32              | [1.02, 3.61]    | 3.14                       | [2.13, 4.15]    | 2.05              | [-0.15, 4.25]    | 1.15                       | [-0.31, 2.62]    |
| GrimAge Clock     | Baseline | 4.65              | [3.73, 5.58]    | 4.98                       | [4.42, 5.54]    | 5.29              | [4.33, 6.25]     | 4.31                       | [3.51, 5.10]     |
|                   | 12mo     | 4.44              | [3.51, 5.38]    | 4.43                       | [3.87, 5.00]    | 5.06              | [4.19, 5.93]     | 4.07                       | [3.36, 4.79]     |
|                   | 24mo     | 3.97              | [3.16, 4.79]    | 4.33                       | [3.72, 4.94]    | 4.88              | [3.88, 5.88]     | 3.50                       | [2.80, 4.19]     |
| PC GrimAge Clock  | Baseline | 12.10             | [11.38, 12.82]  | 12.81                      | [12.24, 13.38]  | 10.15             | [9.08, 11.21]    | 9.65                       | [9.00, 10.29]    |
|                   | 12mo     | 11.69             | [10.85, 12.54]  | 12.35                      | [11.81, 12.88]  | 10.17             | [9.00, 11.35]    | 9.22                       | [8.50, 9.94]     |
|                   | 24mo     | 11.28             | [10.54, 12.03]  | 12.11                      | [11.55, 12.67]  | 9.70              | [8.61, 10.79]    | 8.85                       | [8.15, 9.55]     |

**Panel C. Pace of Aging**

|              |          |      |              |      |              |      |              |      |              |
|--------------|----------|------|--------------|------|--------------|------|--------------|------|--------------|
| Dunedin PACE | Baseline | 0.94 | [0.92, 0.96] | 0.95 | [0.93, 0.96] | 0.94 | [0.92, 0.96] | 0.95 | [0.93, 0.96] |
|              | 12mo     | 0.95 | [0.93, 0.97] | 0.93 | [0.92, 0.94] | 0.95 | [0.93, 0.97] | 0.93 | [0.92, 0.94] |
|              | 24mo     | 0.95 | [0.93, 0.97] | 0.93 | [0.92, 0.95] | 0.95 | [0.93, 0.97] | 0.93 | [0.92, 0.95] |

Table entries for measurements included in primary analysis (PC PhenoAge, PC GrimAge, DunedinPACE) are highlighted in black text.

**Table S8. Change scores for DNA-methylation measures of aging at 12-and 24-month follow-ups for women and men in the CALERIE™ Trial.** Data are the same as reported in Table S3 disaggregated by sex. Panel A shows values for original and PC-based versions<sup>18</sup> of first-generation clocks. Panel B shows values for original and PC-based versions of second-generation clocks. Panel C shows values for DunedinPACE.

**Panel A. First-Generation Clocks**

|                 |      | Women             |                |                            |                | Men               |                |                            |                |
|-----------------|------|-------------------|----------------|----------------------------|----------------|-------------------|----------------|----------------------------|----------------|
|                 |      | Ad Libitum (n=48) |                | Calorie Restriction (n=89) |                | Ad Libitum (n=21) |                | Calorie Restriction (n=39) |                |
|                 |      | Mean              | 95% CI         | Mean                       | 95% CI         | Mean              | 95% CI         | Mean                       | 95% CI         |
| Horvath         | 12mo | -0.02             | [-0.21, 0.17]  | 0.00                       | [-0.15, 0.16]  | -0.21             | [-0.46, 0.04]  | -0.07                      | [-0.26, 0.12]  |
| Clock           | 24mo | -0.04             | [-0.24, 0.17]  | -0.08                      | [-0.22, 0.07]  | -0.24             | [-0.53, 0.05]  | -0.03                      | [-0.20, 0.13]  |
| PC Horvath      | 12mo | -0.16             | [-0.15, 0.01]  | -0.05                      | [-0.10, 0.01]  | -0.09             | [-0.20, 0.01]  | -0.12                      | [-0.21, -0.03] |
| Clock           | 24mo | 0.00              | [-0.24, -0.08] | -0.06                      | [-0.11, -0.01] | -0.11             | [-0.21, 0.00]  | -0.21                      | [-0.29, -0.12] |
| Hannum          | 12mo | -0.07             | [-0.25, 0.11]  | -0.07                      | [-0.21, 0.07]  | -0.18             | [-0.44, 0.07]  | -0.13                      | [-0.35, 0.09]  |
| Clock           | 24mo | -0.02             | [-0.19, 0.15]  | -0.03                      | [-0.17, 0.11]  | -0.14             | [-0.45, 0.18]  | -0.13                      | [-0.37, 0.11]  |
| PC Hannum       | 12mo | -0.06             | [-0.17, 0.05]  | -0.04                      | [-0.12, 0.05]  | -0.07             | [-0.22, 0.07]  | -0.14                      | [-0.27, 0.00]  |
| Clock           | 24mo | -0.17             | [-0.29, -0.05] | -0.04                      | [-0.12, 0.04]  | -0.05             | [-0.20, 0.11]  | -0.21                      | [-0.34, -0.09] |
| Skin & Blood    | 12mo | 0.15              | [-0.04, 0.33]  | 0.06                       | [-0.07, 0.19]  | 0.12              | [-0.13, 0.38]  | -0.04                      | [-0.27, 0.18]  |
| Clock           | 24mo | 0.22              | [0.03, 0.40]   | 0.00                       | [-0.12, 0.12]  | 0.06              | [-0.27, 0.39]  | 0.01                       | [-0.15, 0.17]  |
| PC Skin & Blood | 12mo | -0.10             | [-0.19, 0.00]  | -0.07                      | [-0.13, -0.01] | -0.10             | [-0.21, 0.02]  | -0.11                      | [-0.22, 0.00]  |
| Clock           | 24mo | -0.22             | [-0.32, -0.12] | -0.09                      | [-0.15, -0.03] | -0.11             | [-0.21, -0.01] | -0.21                      | [-0.29, -0.12] |

**Panel B. Second-Generation Clocks**

|             |      | Women             |                |                            |                | Men               |               |                            |                |
|-------------|------|-------------------|----------------|----------------------------|----------------|-------------------|---------------|----------------------------|----------------|
|             |      | Ad Libitum (n=48) |                | Calorie Restriction (n=89) |                | Ad Libitum (n=21) |               | Calorie Restriction (n=39) |                |
|             |      | Mean              | 95% CI         | Mean                       | 95% CI         | Mean              | 95% CI        | Mean                       | 95% CI         |
| PhenoAge    | 12mo | -0.06             | [-0.25, 0.12]  | 0.04                       | [-0.13, 0.21]  | 0.14              | [-0.23, 0.50] | -0.11                      | [-0.31, 0.08]  |
| Clock       | 24mo | -0.18             | [-0.45, 0.08]  | 0.04                       | [-0.10, 0.18]  | -0.04             | [-0.28, 0.21] | -0.01                      | [-0.20, 0.18]  |
| PC PhenoAge | 12mo | -0.05             | [-0.22, 0.12]  | -0.04                      | [-0.16, 0.08]  | 0.05              | [-0.13, 0.24] | -0.12                      | [-0.30, 0.07]  |
| Clock       | 24mo | -0.16             | [-0.34, 0.03]  | 0.05                       | [-0.06, 0.15]  | 0.11              | [-0.11, 0.32] | -0.17                      | [-0.32, -0.02] |
| GrimAge     | 12mo | -0.07             | [-0.21, 0.07]  | -0.21                      | [-0.32, -0.10] | -0.08             | [-0.26, 0.10] | -0.14                      | [-0.32, 0.05]  |
| Clock       | 24mo | -0.27             | [-0.42, -0.13] | -0.21                      | [-0.33, -0.09] | -0.15             | [-0.36, 0.06] | -0.28                      | [-0.43, -0.12] |
| PC GrimAge  | 12mo | -0.13             | [-0.25, -0.01] | -0.16                      | [-0.25, -0.07] | -0.01             | [-0.17, 0.15] | -0.18                      | [-0.33, -0.03] |
| Clock       | 24mo | -0.30             | [-0.44, -0.15] | -0.18                      | [-0.27, -0.10] | -0.16             | [-0.32, 0.00] | -0.29                      | [-0.41, -0.17] |

**Panel C. Pace of Aging**

|         |      |      |               |       |                |      |               |       |               |
|---------|------|------|---------------|-------|----------------|------|---------------|-------|---------------|
| Dunedin | 12mo | 0.07 | [-0.05, 0.31] | -0.16 | [-0.29, -0.02] | 0.14 | [-0.08, 0.36] | -0.22 | [-0.46, 0.02] |
| PACE    | 24mo | 0.00 | [-0.08, 0.23] | -0.09 | [-0.22, 0.04]  | 0.23 | [-0.04, 0.51] | -0.20 | [-0.40, 0.01] |

Table entries for measurements included in primary analysis (PC PhenoAge, PC GrimAge, DunedinPACE) are highlighted in black text.

**Table S9. Effect-estimates from intent-to-treat (ITT) analysis for women and men in the CALERIE™ Trial.** The table shows results from ITT analysis stratified by sex and p-values for tests of sex differences in treatment effects. Tests of sex differences were conducted by including a product term testing interaction between treatment group and sex in the ITT models reported in **Table S4**. Panel A shows treatment effects for original and PC-based versions<sup>18</sup> of first-generation clocks. Panel B shows treatment effects for original and PC-based versions of second-generation clocks. Panel C shows treatment effects for DunedinPACE. All tests reported were two-sided.

**Panel A. First-Generation Clocks**

|                       |      | Women (AL n=48, CR n=89) |                |         |  | Men (AL n=21, CR n=39) |                |         |                                    |
|-----------------------|------|--------------------------|----------------|---------|--|------------------------|----------------|---------|------------------------------------|
|                       |      | Treatment Effect         |                |         |  | Treatment Effect       |                |         |                                    |
|                       |      | (Cohen's D)              | 95% CI         | p-value |  | (Cohen's D)            | 95% CI         | p-value | p-value for test of sex difference |
| Horvath Clock         | 12mo | 0.03                     | [-0.18 , 0.25] | 0.763   |  | -0.03                  | [-0.26 , 0.19] | 0.787   | 0.282                              |
|                       | 24mo | 0.15                     | [-0.19 , 0.48] | 0.389   |  | 0.23                   | [-0.10 , 0.56] | 0.178   | 0.505                              |
| PC Horvath Clock      | 12mo | 0.01                     | [-0.08 , 0.11] | 0.758   |  | 0.09                   | [-0.01 , 0.18] | 0.073   | 0.231                              |
|                       | 24mo | 0.00                     | [-0.12 , 0.13] | 0.943   |  | -0.07                  | [-0.20 , 0.06] | 0.274   | 0.082                              |
| Hannum Clock          | 12mo | 0.01                     | [-0.22 , 0.24] | 0.934   |  | -0.01                  | [-0.22 , 0.19] | 0.895   | 0.741                              |
|                       | 24mo | 0.07                     | [-0.22 , 0.36] | 0.630   |  | 0.04                   | [-0.33 , 0.40] | 0.843   | 0.961                              |
| PC Hannum Clock       | 12mo | 0.02                     | [-0.12 , 0.16] | 0.796   |  | 0.12                   | [-0.02 , 0.27] | 0.101   | 0.125                              |
|                       | 24mo | -0.03                    | [-0.20 , 0.14] | 0.722   |  | -0.14                  | [-0.32 , 0.04] | 0.127   | 0.069                              |
| Skin & Blood Clock    | 12mo | -0.09                    | [-0.30 , 0.13] | 0.434   |  | -0.20                  | [-0.42 , 0.02] | 0.068   | 0.583                              |
|                       | 24mo | -0.10                    | [-0.40 , 0.21] | 0.534   |  | 0.00                   | [-0.35 , 0.35] | 0.999   | 0.347                              |
| PC Skin & Blood Clock | 12mo | 0.02                     | [-0.09 , 0.13] | 0.721   |  | 0.12                   | [0.01 , 0.24]  | 0.037   | 0.256                              |
|                       | 24mo | 0.02                     | [-0.12 , 0.17] | 0.747   |  | -0.06                  | [-0.19 , 0.06] | 0.313   | 0.033                              |

**Panel B. Second-Generation Clocks**

|                |      | Women (AL n=48, CR n=89) |                |         |  | Men (AL n=21, CR n=39) |                |         |                                    |
|----------------|------|--------------------------|----------------|---------|--|------------------------|----------------|---------|------------------------------------|
|                |      | Treatment Effect         |                |         |  | Treatment Effect       |                |         |                                    |
|                |      | (Cohen's D)              | 95% CI         | p-value |  | (Cohen's D)            | 95% CI         | p-value | p-value for test of sex difference |
| PhenoAge Clock | 12mo | 0.13                     | [-0.11 , 0.37] | 0.300   |  | 0.21                   | [-0.05 , 0.46] | 0.112   | 0.201                              |
|                | 24mo | -0.17                    | [-0.54 , 0.20] | 0.361   |  | 0.07                   | [-0.22 , 0.35] | 0.640   | 0.494                              |
| PhenoAge Clock | 12mo | 0.00                     | [-0.19 , 0.20] | 0.996   |  | 0.18                   | [-0.02 , 0.37] | 0.072   | 0.054                              |
|                | 24mo | -0.11                    | [-0.37 , 0.14] | 0.385   |  | -0.24                  | [-0.48 , 0.01] | 0.059   | 0.083                              |
| GrimAge Clock  | 12mo | -0.12                    | [-0.28 , 0.05] | 0.172   |  | 0.06                   | [-0.10 , 0.22] | 0.488   | 0.585                              |
|                | 24mo | -0.04                    | [-0.27 , 0.19] | 0.723   |  | -0.15                  | [-0.38 , 0.08] | 0.193   | 0.098                              |
| GrimAge Clock  | 12mo | 0.00                     | [-0.14 , 0.14] | 0.988   |  | 0.12                   | [-0.03 , 0.26] | 0.117   | 0.089                              |
|                | 24mo | -0.14                    | [-0.35 , 0.07] | 0.187   |  | -0.10                  | [-0.30 , 0.09] | 0.292   | 0.558                              |

**Panel C. Pace of Aging**

|         |      |       |                 |       |  |       |                 |       |       |
|---------|------|-------|-----------------|-------|--|-------|-----------------|-------|-------|
| Dunedin | 12mo | -0.24 | [-0.44 , -0.05] | 0.015 |  | -0.15 | [-0.34 , 0.04]  | 0.114 | 0.166 |
| PACE    | 24mo | -0.39 | [-0.69 , -0.09] | 0.010 |  | -0.46 | [-0.74 , -0.17] | 0.002 | 0.344 |

Table entries for measurements included in primary analysis (PC PhenoAge, PC GrimAge, DunedinPACE) are highlighted in black text.

**Table S10. Effect-estimates from treatment-effect-on-the-treated (TOT) analysis for women and men in the CALERIE™ Trial.** The table shows results from TOT analysis stratified by sex and p-values for tests of sex differences in treatment effects. Tests of sex differences were conducted by including a product term testing interaction between treatment group and sex in the TOT models reported in **Table S4**. Panel A shows treatment effects for original and PC-based versions<sup>18</sup> of first-generation clocks. Panel B shows treatment effects for original and PC-based versions of second-generation clocks. Panel C shows treatment effects for DunedinPACE. All tests reported were two-sided.

**Panel A. First-Generation Clocks**

|                       |      | Women (AL n=48, CR n=89)     |               |         | Men (AL n=21, CR n=39)       |               |         |
|-----------------------|------|------------------------------|---------------|---------|------------------------------|---------------|---------|
|                       |      | Treatment Effect (Cohen's D) | 95% CI        | p-value | Treatment Effect (Cohen's D) | 95% CI        | p-value |
| Horvath Clock         | 12mo | 0.05                         | [-0.29, 0.38] | 0.776   | 0.25                         | [-0.10, 0.61] | 0.154   |
|                       | 24mo | -0.09                        | [-0.49, 0.30] | 0.646   | 0.43                         | [-0.04, 0.89] | 0.072   |
| PC Horvath Clock      | 12mo | 0.09                         | [-0.07, 0.24] | 0.267   | 0.03                         | [-0.10, 0.16] | 0.604   |
|                       | 24mo | 0.16                         | [-0.03, 0.34] | 0.099   | -0.08                        | [-0.31, 0.14] | 0.463   |
| Hannum Clock          | 12mo | 0.07                         | [-0.29, 0.42] | 0.707   | 0.15                         | [-0.21, 0.50] | 0.417   |
|                       | 24mo | 0.05                         | [-0.36, 0.45] | 0.823   | -0.01                        | [-0.57, 0.56] | 0.979   |
| PC Hannum Clock       | 12mo | 0.08                         | [-0.15, 0.30] | 0.512   | -0.05                        | [-0.25, 0.15] | 0.600   |
|                       | 24mo | 0.20                         | [-0.07, 0.48] | 0.151   | -0.22                        | [-0.52, 0.07] | 0.131   |
| Skin & Blood Clock    | 12mo | -0.06                        | [-0.41, 0.29] | 0.742   | 0.01                         | [-0.37, 0.38] | 0.963   |
|                       | 24mo | -0.33                        | [-0.78, 0.12] | 0.151   | -0.12                        | [-0.63, 0.39] | 0.651   |
| PC Skin & Blood Clock | 12mo | 0.08                         | [-0.09, 0.26] | 0.349   | 0.03                         | [-0.15, 0.20] | 0.774   |
|                       | 24mo | 0.17                         | [-0.04, 0.38] | 0.113   | -0.09                        | [-0.29, 0.12] | 0.396   |

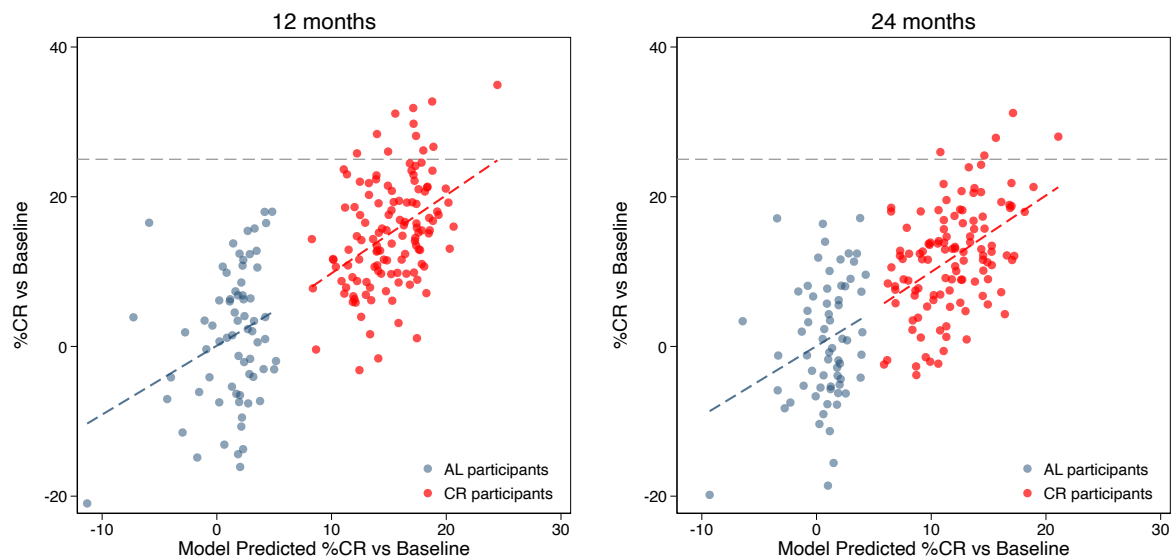
**Panel B. Second-Generation Clocks**

|                   |      | Women (AL n=48, CR n=89)     |               |         | Men (AL n=21, CR n=39)       |                |         |
|-------------------|------|------------------------------|---------------|---------|------------------------------|----------------|---------|
|                   |      | Treatment Effect (Cohen's D) | 95% CI        | p-value | Treatment Effect (Cohen's D) | 95% CI         | p-value |
| PhenoAge Clock    | 12mo | 0.16                         | [-0.20, 0.53] | 0.385   | -0.26                        | [-0.63, 0.11]  | 0.165   |
|                   | 24mo | 0.31                         | [-0.15, 0.76] | 0.185   | 0.07                         | [-0.38, 0.52]  | 0.765   |
| PC PhenoAge Clock | 12mo | 0.01                         | [-0.32, 0.34] | 0.966   | -0.17                        | [-0.49, 0.15]  | 0.303   |
|                   | 24mo | 0.30                         | [-0.05, 0.65] | 0.092   | -0.40                        | [-0.76, -0.04] | 0.028   |
| GrimAge Clock     | 12mo | -0.27                        | [-0.55, 0.01] | 0.056   | -0.08                        | [-0.36, 0.21]  | 0.597   |
|                   | 24mo | 0.01                         | [-0.32, 0.34] | 0.952   | -0.45                        | [-0.81, -0.08] | 0.017   |
| PC GrimAge Clock  | 12mo | -0.02                        | [-0.25, 0.21] | 0.855   | -0.25                        | [-0.51, 0.01]  | 0.061   |
|                   | 24mo | 0.22                         | [-0.03, 0.47] | 0.091   | -0.30                        | [-0.58, -0.03] | 0.030   |

**Panel C. Pace of Aging**

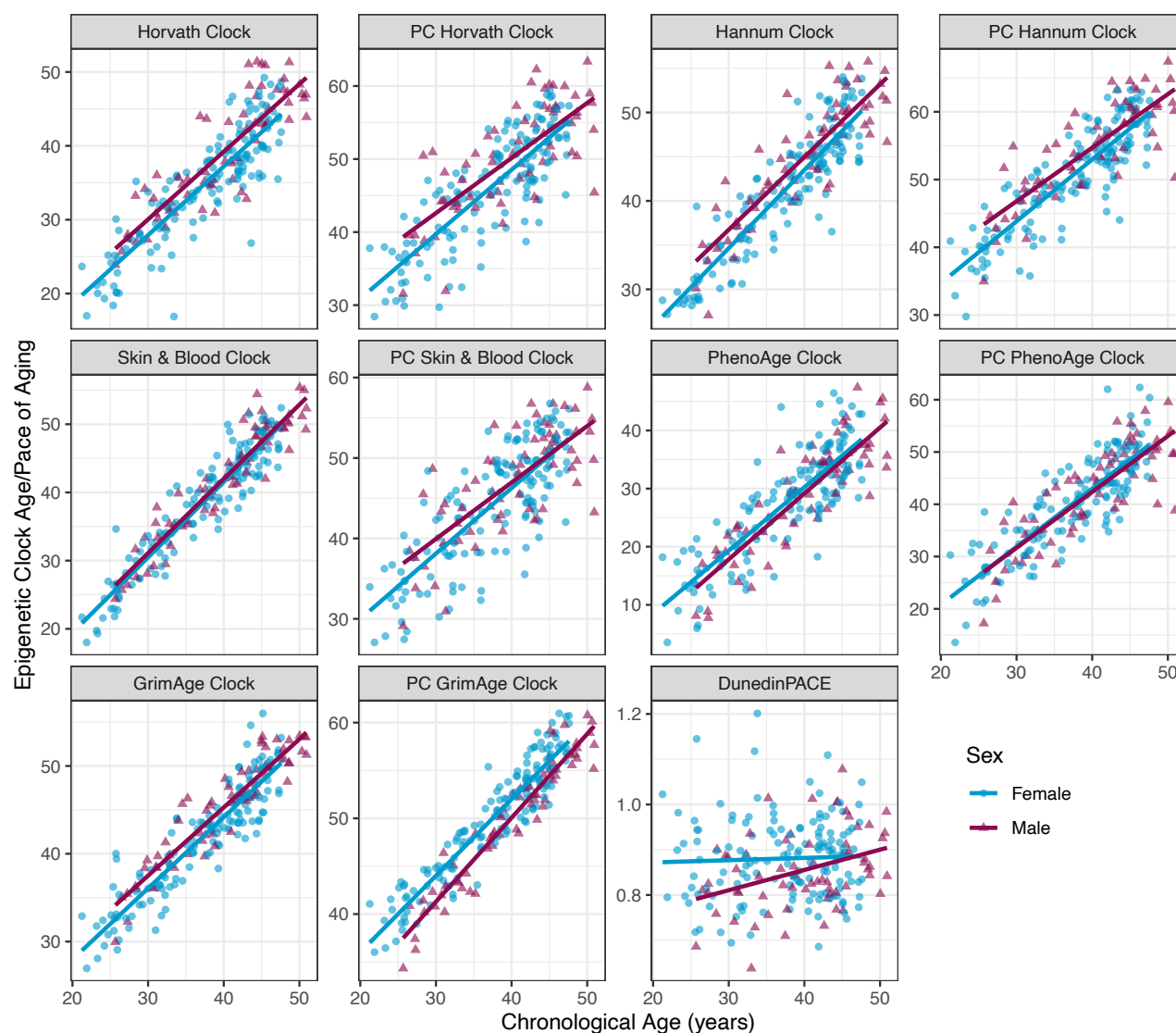
|              |      |              |                       |              |              |                       |              |
|--------------|------|--------------|-----------------------|--------------|--------------|-----------------------|--------------|
| Dunedin PACE | 12mo | <b>-0.42</b> | <b>[-0.73, -0.11]</b> | <b>0.007</b> | <b>-0.59</b> | <b>[-0.93, -0.25]</b> | <b>0.001</b> |
|              | 24mo | -0.32        | [-0.69, 0.04]         | 0.081        | <b>-0.76</b> | <b>[-1.21, -0.30]</b> | <b>0.001</b> |

Table entries for measurements included in primary analysis (PC PhenoAge, PC GrimAge, DunedinPACE) are highlighted in black text. Bolded entries fall below the significance threshold of  $p < 0.005$ .



**Figure S1. Actual %CR plotted against Predicted CR.** The figure plots the measured % CR vs baseline (y-axis) against the model-predicted CR (x-axis) at the 12- (left) and 24-month (right) follow-up assessments. Values for ad-libitum (AL) control-group participants are plotted in blue. Values for caloric restriction (CR) intervention-group participants are plotted in red. The intervention target of 25% CR is noted with a dashed gray line intersecting the y-axis. Predicted values are derived from the base model for the first stage of IV regression analysis described in equation (1). The model includes terms for intervention condition, sex, pre-treatment baseline body-mass index, interactions among these variables, and covariates for pre-treatment baseline chronological age, study site, and race/ethnicity.

**Figure S2. Associations of DNA methylation measures of aging with chronological age at pre-intervention baseline in the CALERIE™ Trial.** Figures show scatter plots of the DNA methylation measures of aging (Y-axis) against chronological age (X-axis) for women (blue circles) and men (purple triangles) and fitted regression slopes. Correlations with chronological age are as follows: Horvath Clock  $r=0.87$ , PC Horvath Clock  $r=0.84$ , Hannum Clock  $r=0.92$ , PC Hannum Clock  $r=0.88$ , Skin & Blood Clock  $r=0.94$ , PC Skin & Blood Clock  $r=0.80$ , PhenoAge Clock  $r=0.84$ , PC PhenoAge Clock  $r=0.85$ , GrimAge Clock  $r=0.93$ , PC GrimAge Clock  $r=0.92$ , DunedinPACE  $r=0.15$ .





**Figure S3. Change from baseline to 12- and 24-month follow-up in DNA methylation measures of aging in the ad libitum (AL) and caloric restriction (CR) groups in the CALERIE™ Trial.** Values for the AL control group (n = 69 participants) are graphed in blue. Values for the CR treatment group (n = 128 participants) are graphed in red. Panel A mean values of change from baseline and 95% confidence intervals estimated from mixed models at the 12- and 24-month follow-ups for the AL and CR groups. There is no confidence interval estimated for baseline because change from baseline is exactly zero at this timepoint. Panel B shows box plots of the observed values of the measures at baseline and 12- and 24-month follow-ups. The boxes show the interquartile range; the whiskers show 1.5x the interquartile range; the center line shows the median; individual participant data are plotted as dots and connected with lines. Change scores are denominated in “years” of DNA methylation age. For the clocks, expected change under the null hypothesis is 1 year at 12-months follow-up and 2 years at 24 months follow-up.

Panel A

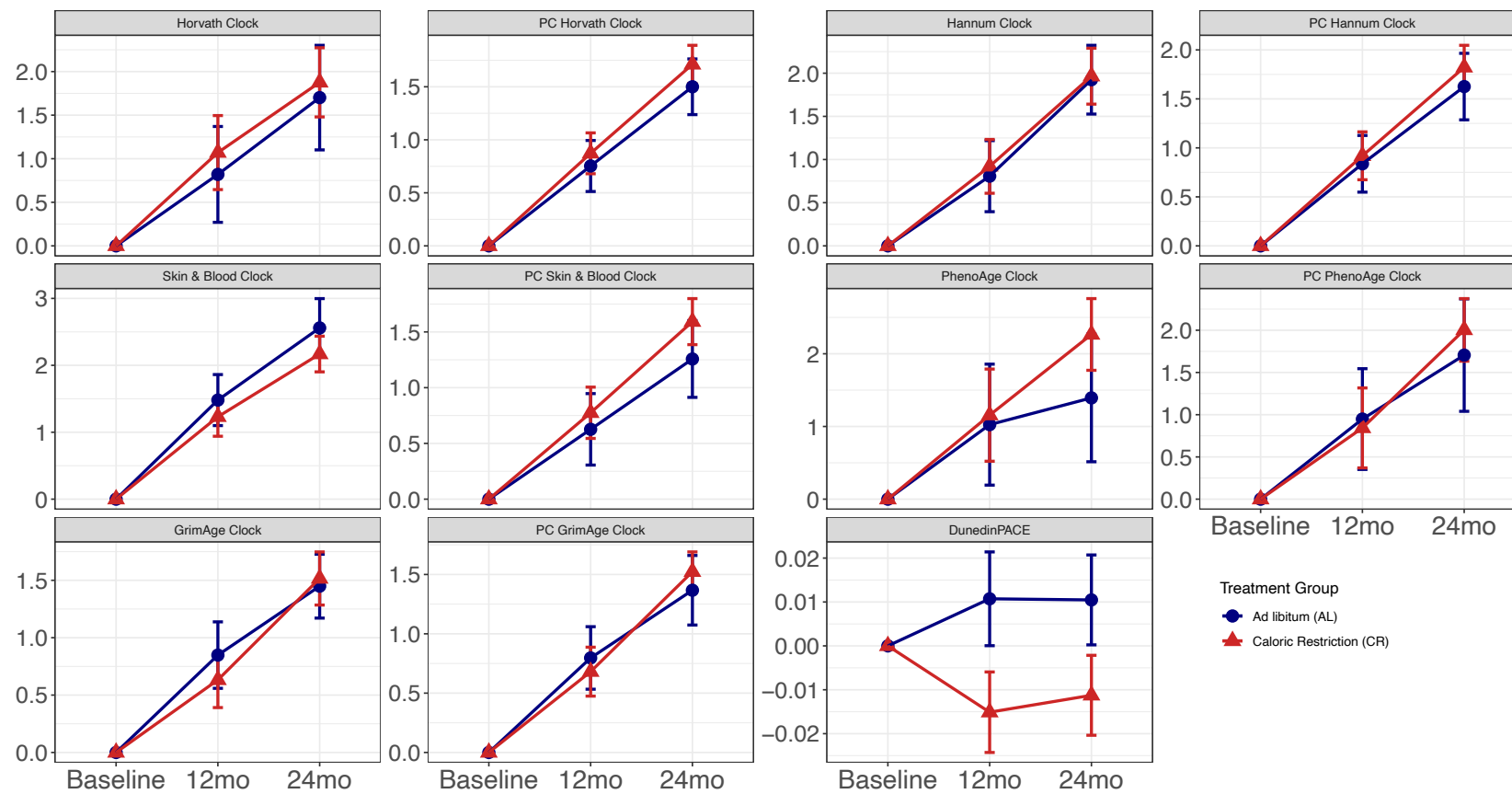
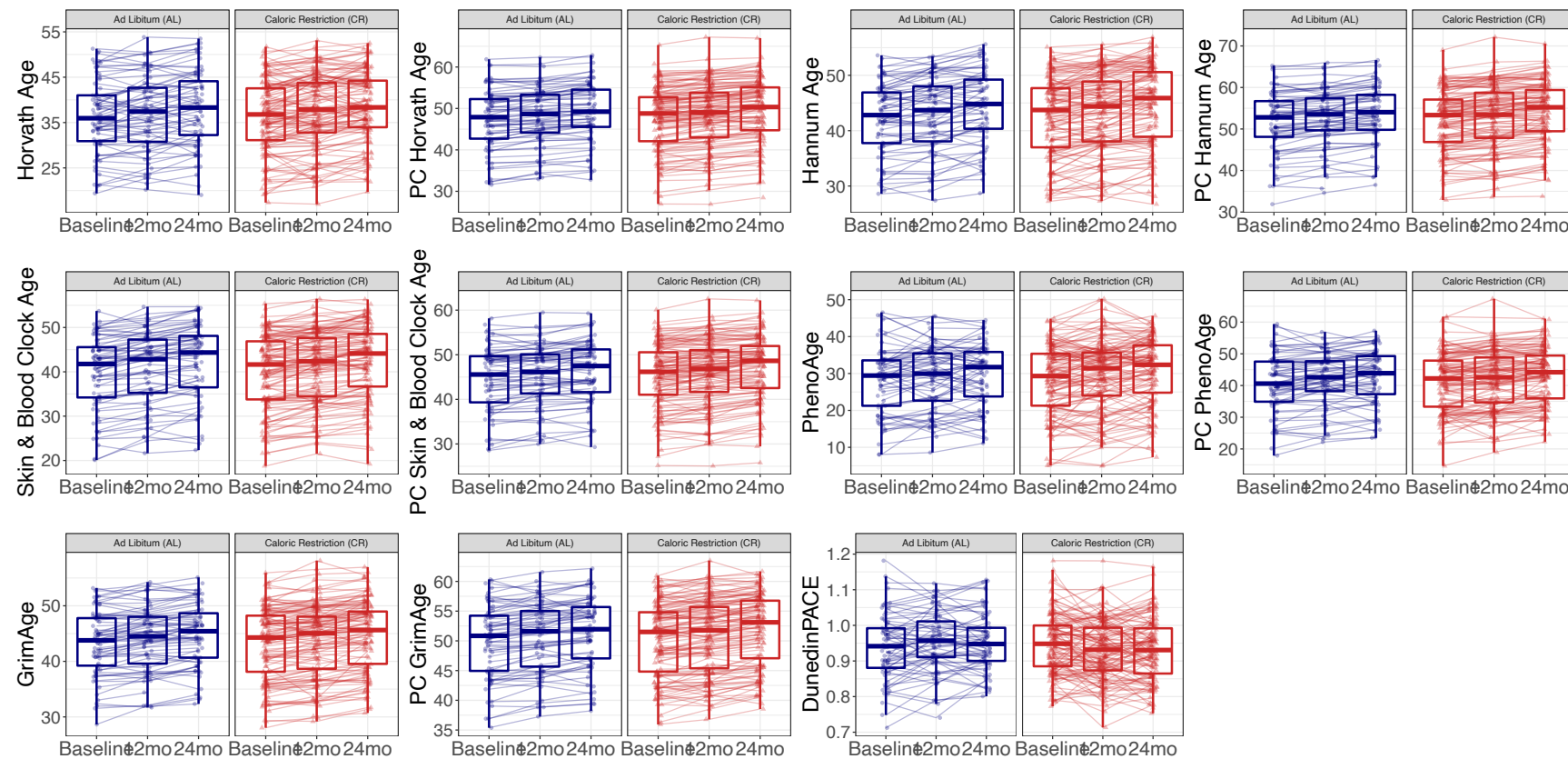


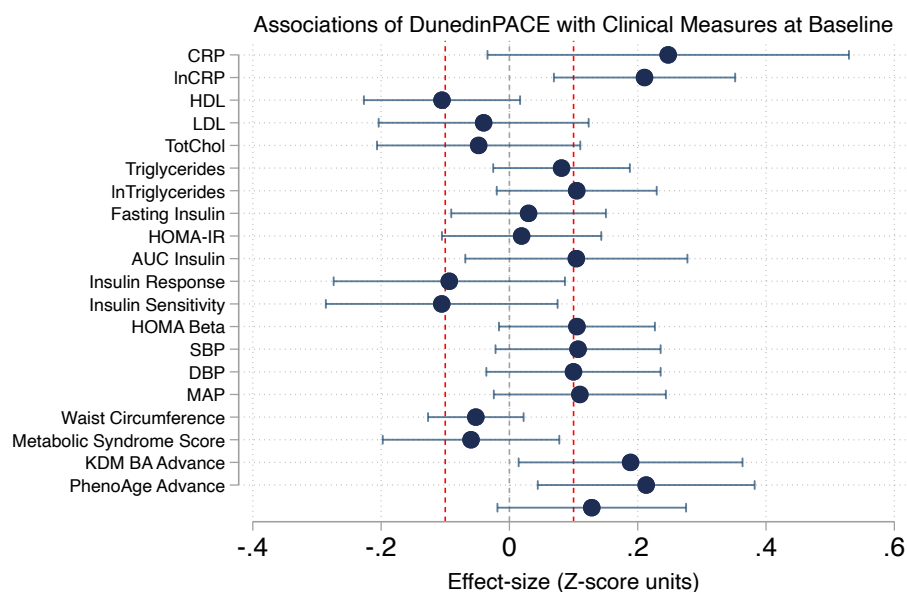
Figure S3 Continued

Panel B



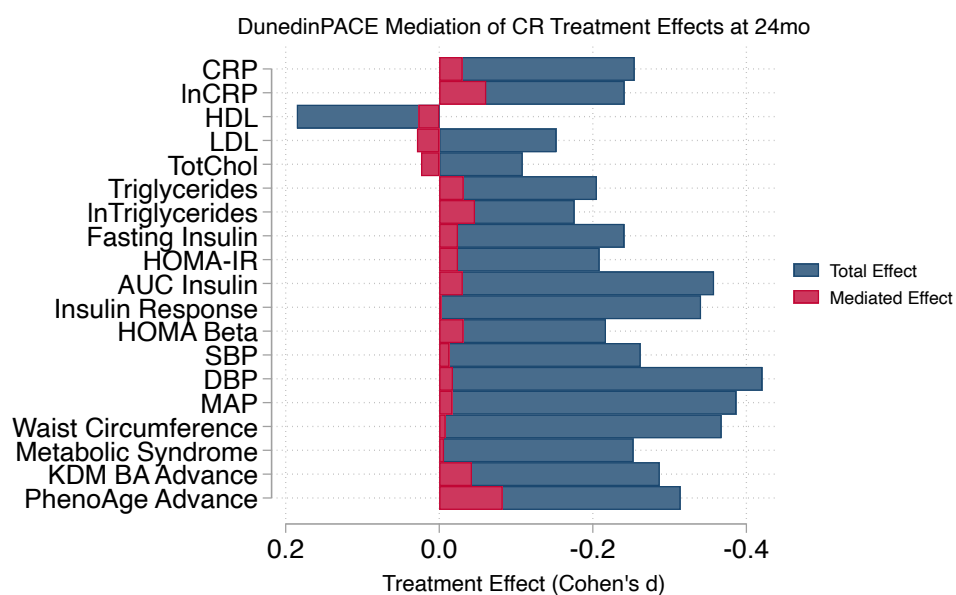
**Figure S4. Association of DunedinPACE with clinical measures modified by CALERIE™ intervention.** To evaluate whether intervention-induced changes in DunedinPACE overlapped with intervention effects on clinical measures, we conducted analysis of clinical measures altered by CALERIE™ intervention as reported in prior publications by our group<sup>2,28</sup>. Panel A shows effect-sizes (and 95% confidence intervals) for associations between DunedinPACE and each of the clinical measures at pre-intervention baseline (n = 197). Effect-sizes are graphed as Pearson r to be comparable across clinical measures with different scales. Panel B graphs estimates of total effects of CALERIE™ treatment on each of the clinical measures (blue bars) and indirect effects mediated through DunedinPACE (red bars). Effect estimates were generated according to the equations published by Imai et al.<sup>29,29</sup> implemented with the Stata package ‘medeff’. Treatment effects are reported on a standard deviation scale (Cohen’s d) so they are comparable between clinical measures. Confidence intervals did not overlap zero for estimates of mediation percent for log CRP, HDL, Triglycerides, log Triglycerides, Insulin AUC, SBP, DBP, Waist Circumference, Metabolic Syndrome, KDM BA, PhenoAge. For estimates of the adjusted causal mediation effect (ACME), confidence intervals overlapped zero for all outcomes with the exception of log-CRP and PhenoAge.

**Panel A.**



**Figure S4 Continued**

**Panel B.**



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