**Appendix A. Flowchart, Whitehall II**

Missing on caregiving (n=263)

Missing on confounders (n=138)

No returned samples (n=857)

Missing CAR samples (n=70)

Wakeup <4am or >11am (n=76)

>3SD cortisol (n=179)

>10 min wakeup measure (n=612)

>45 min awakening response (n=40)

Corticosteroids (n=1)

Participants in phase 9 (n=6,761)

Agreed to participate in cortisol collection (n=5,963)

Included in analyses (n=3,727)

Valid cortisol data (n=4,128)

**Appendix B. Directed acyclic graph of the causal effect of informal caregiving on cortisol levels**

 

**Appendix C. Sub- and sensitivity analyses**

|  |  |  |
| --- | --- | --- |
|  | Men (n=2,772) | Women (n=955) |
|  | **Cortisol at wake-up** | **Cortisol at****30 min** | **Cortisol at****bedtime** | **CAR** | **Cortisol at wake-up** | **Cortisol at****30 min** | **Cortisol at****bedtime** | **CAR** |
| Informal caregiving: |  |  |  |  |  |  |  |  |
| Caregivers | 15.0 (±7.3) | 19.9 (±10.2) | 2.3 (±2.2) | 4.9 (±10.1) | 13.6 (±6.6) | 22.6 (±13.0) | 2.9 (±3.0) | 9.0 (±12.7) |
| Non-caregivers | 14.9 (±7.3) | 21.3 (±11.4) | 2.5 (±2.4) | 6.4 (±11.1) | 13.9 (±7.8) | 21.8 (±11.8) | 2.5 (±2.1) | 7.9 (±11.6) |
| Care recipient: |  |  |  |  |  |  |  |  |
| Spouse | 14.6 (±6.4) | 19.1 (±8.8) | 2.5 (±1.9) | 4.5 (±9.1) | 12.5 (±6.0) | 24.1 (±14.7) | 2.8 (±2.5) | 11.7 (±12.7) |
| Parent, relative or friend | 15.1 (±7.7) | 20.3 (±10.8) | 2.2 (±2.4) | 5.1 (±10.5) | 14.1 (±6.8) | 22.0 (±12.3) | 3.0 (±2.1) | 8.0 (±12.6) |
| Weekly caregiving hours: |  |  |  |  |  |  |  |  |
| >100 hours | 16.0 (±8.8) | 20.0 (±7.8) | 1.9 (±1.2) | 4.0 (±8.5) | 12.5 (±6.3) | 21.3 (±14.9) | 3.2 (±3.2) | 8.8 (±12.7) |
| 20-100 hours | 16.7 (±7.7) | 20.0 (±9.3) | 2.0 (±1.5) | 3.3 (±10.0) | 13.2 (±8.2) | 22.7 (±12.7) | 2.8 (±2.7) | 9.5 (±14.8) |
| 9-20 hours | 14.4 (±7.6) | 19.3 (±11.2) | 2.4 (±3.1) | 4.9 (±10.3) | 12.7 (±6.9) | 21.0 (±13.4) | 3.6 (±4.5) | 8.3 (±12.5) |
| 1-8 hours | 14.7 (±6.2) | 20.7 (±10.3) | 2.4 (±2.2) | 6.0 (±10.3) | 14.5 (±5.8) | 24.5 (±12.8) | 2.4 (±2.3) | 10.0 (±12.2) |
| Duration of caregiving: |  |  |  |  |  |  |  |  |
| Long-term caregiving | 15.3 (±7.4) | 20.7 (±10.2) | 2.0 (±1.4) | 5.5 (±9.8) | 13.7 (±7.0) | 23.5 (±14.0) | 3.0 (±2.9) | 9.8 (±13.0) |
| Short-term caregiving | 14.8 (±7.6) | 17.9 (±9.2) | 2.5 (±3.0) | 3.1 (±9.5) | 14.8 (±6.7) | 22.6 (±12.0) | 3.2 (±3.5) | 7.8 (±12.9) |
| Informal caregiving and paid work: |  |  |  |  |  |  |  |  |
| Caregivers, in paid work | 16.2 (±8.2) | 19.6 (±9.9) | 2.1 (±1.6) | 3.3 (±11.3) | 14.8 (±8.2) | 23.5 (±8.7) | 2.3 (±1.6) | 8.7 (±9.4) |
| Caregivers, not in paid work | 14.4 (±6.9) | 20.0 (±10.3) | 2.3 (±2.5) | 5.6 (±9.4) | 13.3 (±6.1) | 22.4 (±14.1) | 3.1 (±3.2) | 9.1 (±13.6) |
| Non-caregivers, in paid work | 14.7 (±7.0) | 21.5 (±11.2) | 2.5 (±2.6) | 6.8 (±11.3) | 14.0 (±6.6) | 21.4 (±12.5) | 2.5 (±2.3 | 7.4 (±12.4) |
| Non-caregivers, not in paid work | 14.9 (±7.5) | 21.2 (±11.6) | 2.5 (±2.4) | 6.3 (±11.0) | 13.9 (±8.1) | 21.9 (±11.6) | 2.5 (±2.1) | 8.1 (±11.3) |

**Appendix C1. Various aspects of informal caregiving and cortisol levels**

Cortisol presented as nmol/L mean (±SD). Abbreviations: CAR = Cortisol Awakening Response

**C2. Unadjusted associations between various aspects of informal caregiving and cortisol awakening response and diurnal cortisol slope**

|  |  |  |
| --- | --- | --- |
|  | **Men (n=2,772)** | **Women (n=955)** |
|  | **Difference in CAR****Unadjusted, β (95%CI)** | **Difference in Slope****Unadjusted, β (95%CI)** | **Difference in CAR****Unadjusted, β (95%CI)** | **Difference in Slope****Unadjusted, β (95%CI)** |
| **Informal caregiving** | -1.633 (-2.991;-0.275) | 0.000 (-0.007;0.008) | 1.205 (-1.034;3.444) | -0.001 (-0.012;0.010) |
| **Care recipient:** |  |  |  |  |
| Spouse | -2.208 (-4.539;0.123) | 0.008 (-0.005;0.020) | 3.676 (-0.246;7.598) | -0.005 (-0.025;0.015) |
| Parent, relative or friend | -1.368 (-2.982;0.246) | -0.003 (-0.012;0.006) | 0.171 (-2.442;2.783) | 0.000 (-0.013;0.013) |
| **Weekly caregiving hours:** |  |  |  |  |
| 1-8 hours | -0.416 (-2.411;1.579) | -0.000 (-0.011 ;0.010) | 1.983 (-1.855;5.821) | -0.016 (-0.035;0.004) |
| 9-20 hours | -1.596 (-4.406;1.214) | 0.010 (-0.005;0.025) | 0.489 (-4.744;5.722) | -0.002 (-0.028;0.023) |
| 20-100 hours | -3.184 (-6.611;0.244) | -0.012 (-0.031;0.006) | 1.518 (-2.926;5.962) | -0.001 (-0.023;0.021) |
| >100 hours | -2.565 (-6.333;1.203) | -0.009 (-0.028;0.011) | 0.862 (-3.745;5.468) | 0.016 (-0.007;0.039) |
| Test for trend | p=0.026 | p=0.476 | p=0.392 | p=0.546 |
| **Duration of caregiving:** |  |  |  |  |
| Long-term caregiving | -1.015 (-2.880;0.850) | -0.009 (-0.019;0.002) | 1.834 (-1.118;4.786) | 0.001 (-0.014;0.016) |
| Short-term caregiving | -3.295 (-5.343;-1.247) | 0.008 (-0.003;0.020) | -1.097 (-4.747;2.552) | 0.004 (-0.016;0.023) |

CAR=Cortisol Awakening Response (nmol/L). Slopes are analysed using log(cortisol). Non-caregivers is the reference group in all analyses. Multiple adjusted: Age, ethnicity, married/cohabiting, gainful employment, income category, wakeup time. For difference in the CAR a negative β represents a blunted response. For difference in cortisol slope a negative β represents a steeper slope.

**Appendix C3. Unadjusted joint effects of informal caregiving and paid work on cortisol awakening response and diurnal cortisol slope**

|  |  |  |
| --- | --- | --- |
|  | **Men (n=2,772)** | **Women (n=955)** |
|  | **Difference in CAR, Unadjusted, β (95%CI)** | **Difference in CAR, Unadjusted, β (95%CI)** |
| Caregivers, workers | -3.227 (-5.657;-0.797) | 0.602 (-3.856;5.059) |
| Caregivers, non-workers | -0.814 (-2.433;0.805) | 1.179 (-1.379;3.737) |
| Non-caregivers, workers | 0.339 (-0.593;1.271) | -0.632 (-2.515;1.251) |
| Non-caregivers, non-workers | 0 (ref.) | 0 (ref.) |
|  | **Difference in cortisol slope****Unadjusted, β (95%CI)** | **Difference in cortisol slope****Unadjusted, β (95%CI)** |
| Caregivers, workers | -0.000 (-0.013;0.013) | -0.015 (-0.038;0.007) |
| Caregivers, non-workers | -0.000 (-0.009;0.009) | 0.005 (-0.008;0.017) |
| Non-caregivers, workers | -0.001 (-0.006;0.004) | 0.004 (-0.005;0.014) |
| Non-caregivers, non-workers | 0 (ref.) | 0 (ref.) |

CAR: Cortisol Awakening Response. Slopes were analyzed using log(cortisol) with hours as the underlying time axis. Multiple adjusted: Age, ethnicity, married/cohabiting, gainful employment, income category, wakeup time. CAR: Interaction, men p=0.07, women p=0.97. Slope: Interaction, men p=0.84, women p=0.01. For difference in the CAR a negative β represents a blunted response, and for the difference in cortisol slope a negative β represents a steeper slope.

**C4. Sensitivity analyses for the association between informal caregiving and various analyses on cortisol awakening response**

Slope exclusion (n=231)

Missing timepoint (n=8)

CAR exclusion (n=545)

Included in slope analyses (n=3,932)

Included in CAR analyses (n=3,626)

|  |  |  |
| --- | --- | --- |
|  | **Men (n=2,772)** | **Women (n=955)** |
|  | **Difference in CAR****Multiple adjusted, β (95%CI)** | **P-value** | **Difference in CAR****Multiple adjusted, β (95%CI)** | **P-value** |
| **Informal caregiving, main analysis** | -1.523 (-2.882;-0.163) | p=0.028 | 1.436 (-0.805;3.678) | p=0.209 |
| **Adjusted for morning cortisol** | -1.480 (-2.784;-0.177) | p=0.026 | 1.363 (-0.781;3.506) | p=0.213 |
| **CAR/time difference** | -0.049 (-0.092;-0.006) | p=0.026 | 0.044 (-0.027;0.114) | p=0.227 |

CAR: Cortisol Awakening Response. Multiple adjusted: Age, ethnicity, married/cohabiting, gainful employment, income category, wakeup time. For difference in the CAR a negative β represents a blunted response.