Relationship of nighttime arousals and nocturnal cortisol in IBS and normal subjects

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Introduction

Both cortisol level changes and sleep disturbances have been associated with many disorders thought to include an element of disordered regulation of the hypothalamic-pituitary-adrenal (HPA) axis, a mediator of stress response.[1–4] However, findings regarding the existence and direction of cortisol changes and sleep disturbances in both healthy controls and subjects with HPA-related disorders have been inconsistent. This study presents the results of an analysis of the relationship between nocturnal cortisol levels and sleep disruption in a sample of women with irritable bowel syndrome (IBS).

Normal cortisol secretion follows a diurnal cycle reaching its lowest level during the first part of the night, followed by a steep rise during the latter part of the sleep cycle.[2] This basal activity is governed by an internal circadian clock, and is pulsatile in nature, with individual peaks detectable throughout the diurnal profile.[3,5–8] When a perceived threat or challenge activates the HPA neuroendocrine stress response system, a cascade is initiated beginning with the release of corticotropin-releasing hormone (CRH) from the hypothalamus and continuing through the release of adrenocorticotropic hormone (ACTH) from the pituitary to the final release of cortisol from the adrenal cortex, superimposed on the basal production.[2–4,9,10] In normal subjects, a stress-related increase in cortisol secretion tends to be followed by a negative-feedback induced compensating decrease, resulting in a homeostatic correction that keeps overall 24-hour cortisol within a normal range.[9] Some factors that have been shown to affect cortisol levels include age, sleep quality, “persisting pain,” and perceived stress, but not BMI.[2,11,12],[13]

There is conflicting evidence regarding cortisol changes in many disorders where HPA dysregulation is known or suspected. Reduced cortisol has been reported in subjects with depression, post-traumatic stress disorder (PTSD), and fibromyalgia (FM), as well as chronic fatigue syndrome (CFS) and chronic pain syndromes.[9],[14],[15], [16], [17], [18] Other studies have found no difference between cortisol levels in controls and subjects with PTSD or CFS.[14], [17] Elevated cortisol has been reported in FM, depression, PTSD, primary insomnia, restless leg syndrome (RLS), and panic disorder, and separately in men with depression and women with comorbid depression and PTSD. [2], [4], [9],[14],[19], [20],[21],[1,22], [23], [24] Some authors have suggested that these discrepancies may be partly explained by a process where prolonged stress may result in overproduction of cortisol (hypercortisolism) in the short term, but eventually lead to decreased cortisol and reduced responsiveness (hypocortisolism).[26–29] This is supported by a finding that among subjects with FM, there was a trend for cortisol levels to be lower with longer duration of disease.[11]

Conflicting results have also been found with regard to sleep disturbances in HPA-related disorders. Depression has been linked to difficulty falling asleep, frequent awakenings and early morning awakening, and reduced sleep efficiency. [2],[20,30],[31] Lower sleep efficiency has also been reported in subjects with attention deficit hyperactivity disorder (ADHD), PTSD, IBS, CFS and FM, compared to healthy controls. [32],[33],[11,34],[35],[36],[37] One study reported a trend for subjects with FM to have more frequent arousals than controls.[38] However, other studies have found no differences in sleep quality or architecture for PTSD subjects compared to controls, and have reported that while self-reported sleep quality was reduced in subjects with IBS, PTSD, and CFS, objective measures of sleep quality did not differ between patients and controls.[14],[33],[39],[40],[17,25] Rather, the self-reported sleep problems and the poor correlation between self-reported and objective sleep symptoms were attributed to a reporting bias on the part of patients suffering from stress-related disorders.[33],[41] A number of studies have examined the relationship between cortisol and sleep in normal subjects, with varied results. One study found that experimental sleep fragmentation resulted in increased morning serum cortisol levels, while another found that experimental awakenings reduced total plasma cortisol and a third found that experimental awakenings first increased, then decreased cortisol, resulting in no net change in overall mean nightly concentration.[7], [42],[45] Some of these discrepancies may be attributable at least in part to intra- and inter-individual variability, both in natural cortisol production and in HPA responsiveness to sleep disruption, as suggested by the finding that approximately 25% of healthy individuals do not display increased cortisol after awakenings.[44,45] Timing of awakenings may be an important factor in the sleep-cortisol relationship; in one study, arousals during the 1st rapid eye movement (REM) period did not induce a cortisol burst, while in another, arousals (stage 1 sleep and awakenings) mostly occurred when cortisol levels were increasing.[8,45] Duration of awakenings may play a role as well, since cortisol increases were found in one study to be associated with prolonged awakenings.[44] Further complicating the picture is evidence suggesting that HPA activation may drive sleep disruption instead of, or in addition to, sleep disruption driving HPA activation. For example, nocturnal cortisol peaks induced by injected ACTH are followed by increased awakenings, experimentally elevated CRH resulted in increasing light sleep and wakefulness, and elevated evening cortisol levels raised the number of awakenings later in the night. [2,15,46],[47],[48],[24]

In subjects with stress-related disorders, varied results have been reported for associations of sleep disruption and cortisol. In subjects with FM, poor sleep has been associated with cortisol levels that were increased, decreased, or unchanged[13],[38] Both nocturnal awakenings and elevated nocturnal cortisol were reported in subjects with PTSD and those with panic disorder, as well as subjects with primary insomnia. [1,22],[21],[24] In RLS, higher nocturnal cortisol was correlated with the number of awakenings.[23] Disturbed sleep and hypocortisolism have been found in subjects with CFS.[49] In depressed subjects, disturbed sleep has been associated with both elevated and reduced nocturnal cortisol.[46],[50]

Additional studies that carefully examine the association between sleep disruption and nocturnal cortisol levels in specific populations are needed to identify where such associations exist and the direction and magnitude of those associations, including whether sleep disruption appears to play a major or minor role in mediating the effect of the disorder on cortisol levels. Examinations of the temporal relationship of nighttime awakenings and nocturnal cortisol levels may also help to clarify the causal relationships between sleep disturbance and HPA activation.

This thesis is a secondary analysis of data from a study of nocturnal cortisol and catecholamine levels in women with IBS and normal controls. Details of the study’s sample and methods have been published in Burr et al., 2009.[51] In brief, 31 healthy controls and 30 women with a diagnosis of IBS spent two acclimatory nights in the sleep laboratory and on the third night had blood samples collected through an intravenous line every 20 minutes, which were analyzed for plasma cortisol levels. Polysomnographic recordings, together with study nurse notes, provided detailed information on sleep stage and arousals throughout the period of sample collection. The primary aim of this thesis is to assess whether sleep disruption plays a mediating role in the relationship between IBS and nocturnal cortisol levels. The secondary aims are to conduct exploratory analyses, first, to assess the effect of additional covariates on the mediation results, and second, to examine the temporal relationship of nocturnal awakenings and cortisol changes.

Methods

Choice of mediation methods

The goal of the mediation analysis was to investigate whether there was an indirect relationship of IBS to nightly cortisol levels mediated through sleep disruption. This was complicated by the nature of the data: multilevel, longitudinal outcomes (multiple cortisol measurements over time for each subject) and a nominal categorical predictor (IBS subtypes). Finding mediation methods to deal with either or both of these issues was a challenge. Traditional mediation methods rely on the predictor variable being continuous or binary, while the only method I could find (based on a paper still in review) to deal with a categorical predictor was not designed to deal with multilevel data. For my primary analysis, I ended up using a mediation method that was a variation of the categorical method, modified to accommodate the longitudinal outcomes. I then conducted sensitivity analyses using the original categorical predictor method and multiple traditional mediation methods.

Description of mediation methods

The categorical predictor method is described in a paper by Hayes and Preacher[1]. The method is based on two models where the categorical predictor with *k* levels is represented by *k*-1 indicator (“dummy”) variables, e.g., $P_1 \ldots P_{k-1}$. The first model has no mediator variable and represents the *total effect* of each predictor indicator on the outcome, Y, as summarized by the pathway diagram $P_i \xrightarrow{c_i} Y$. The effects are numbered to match the indicator variables, $c_1 \ldots c_{k-1}$. The second model contains a mediator variable, M, so that the predictor may be associated directly with the outcome as above ($P_i \xrightarrow{c'_i} Y$) or indirectly through the mediator, with a two-step pathway: $P_i \xrightarrow{a_i} M \xrightarrow{b} Y$. The pathways representing the effects of the predictor indicators on the mediator are again numbered to match the indicators, $a_1 \dots a_{k-1}$. However, in this model there is only a single pathway, b, representing the effect of the mediator on the outcome (this assumes that the mediator is not also categorical; if it were, a more complicated model with multiple b pathways would be necessary). The total effect of the predictor indicators on the outcome is divided between *direct effects*, which do not involve the mediator, and *indirect effects*, which involve the two-step pathway traveling from predictor through mediator to outcome. The direct effects are labeled $c'_1 \dots c'_{k-1}$ to distinguish them from the total effects, $c_1 \dots c_{k-1}$.

As described in the Hayes and Preacher paper, the indirect effects are calculated as the products of coefficients in each of the indirect pathways, $a_1{*}b \dots a_{k-1}{*}b$.[1] The mediator acts as the outcome of the first step pathway, $P_i \xrightarrow{a_i} M$, and as the predictor of the second step pathway, $M \xrightarrow{b} Y$. This is straightforward in the case of data that are not multilevel, for example where there is a single observation per person. It is less straightforward in the case of multilevel data where P and M are at different levels from Y. Consider the longitudinal IBS data: the outcome Y is nightly serum cortisol values, measured repeatedly during the time after entering stage 2 sleep, with up to 6 observations per subject. Any model using these cortisol values as the outcome must take into account the fact that individual cortisol values (level 1) are nested within subjects (level 2) as well as the fact that cortisol levels change in a nonlinear fashion over time during the night. By contrast, both the predictor of interest, IBS subtype, and the mediator, a summary of sleep disruption, are person-level (level 2) variables. The $P_i \xrightarrow{a_i} M$ model thus has only a single observation per subject with no nesting or time structure.

An alternative way to calculate the indirect effects is to calculate the difference between the effect of the predictor indicator on the outcome with no mediator in the model – the total effect, c_i – and the direct effect, c'_i, of the predictor indicator on the outcome, from the model with mediator included.[2] In this case, both coefficients, c_i and c'_i, come from models with the same structure; the only difference is that an extra variable, M, is added to the c'_i model. I used this alternative "difference of coefficients" way of calculating the indirect effects in my primary analysis. As a sensitivity analysis, I compared the results obtained by calculating the indirect effect using the "product of coefficients" ($a_\mathrm{i}*b$) and "difference of coefficients" (c_i - c'_i) methods.

For additional sensitivity analyses, I used binary predictors comparing all IBS subjects to controls, comparing individual IBS subtypes to controls, or comparing IBS-constipation to IBS-diarrhea. I also tried several mediation methods developed for continuous or binary predictors. In such methods, there are two models as described above, one with and one without a mediator, but only one coefficient for each pathway leading from the predictor. Thus the total effect is given by c in P $\xrightarrow{c}$ Y, the direct effect is given by c' in P $\xrightarrow{c'}$ Y, and the indirect effect is given by $a*b$ (from the two steps P $\xrightarrow{a}$ M $\xrightarrow{b}$ Y) or by c- c'.

Whether the predictor is coded with dummy variables, a binary variable, or a multilevel variable changes the interpretation of the effects. With dummy variables, the i^{th} effect is interpreted as a comparison of the i^{th} predictor category to the reference category. For a binary variable, the effect is interpreted simply as the effect of the predictor being present, compared to its absence. For a multilevel variable, the effect is interpreted as the effect of a one-unit increase in the variable, which is entirely dependent on the coding of the levels. If there is no sensible way to order the levels, or if the difference between levels is highly variable, the interpretation of the effect may not be meaningful. The constipation-predominant, diarrhea-predominant, and alternating IBS subtypes are not ordered categories, so I did not use multilevel variable coding.

Choice of mediator variable

The question of interest is whether IBS has an indirect effect on nocturnal cortisol levels, mediated through nighttime arousals. Sleep disruption and arousals can be quantified in many ways and it is not clear a priori whether frequency, duration, timing, or some combination of these is most likely to be important as a mediator. As a first step toward investigating such a complex topic, either duration or frequency of arousals seem like reasonable choices. I decided to use a measure of duration, percent sleep period in stage 0 or 1, in my primary analysis and to conduct a sensitivity analysis comparing that mediator variable to the fragmentation index, a per-hour index (rate) of arousal frequency, calculated by dividing each subject's total arousals (from sleep stage 2-5 to 0 or 1) by total sleep time.

Inference for mediation analysis

In their paper developing a method for mediation analysis using categorical predictors, Hayes and Preacher discuss inference for total, direct and indirect effects, either singly, in combination or as a class[1]. In this case, there is no a priori hypothesis about any particular effect or predictor category. Rather, the question is a general one of identifying whether any indirect effect exists through the pathways $\begin{matrix}\text{constipation}\\ \text{diarrhea}\\ \text{alternating}\end{matrix} \rightarrow$ arousals → cortisol. This leads to the null hypothesis that no such indirect effect exists, or equivalently that all the indirect effects equal 0. Hayes and

Preacher describe this as “omnibus inference.” They suggest that one approach to conducting such a test is to use “Bonferroni-corrected asymmetric confidence intervals,” where a single confidence interval excluding 0 is sufficient evidence to reject the null hypothesis of no indirect effect. The adjusted confidence level is set at $100[1 - \frac{\propto}{k-1}]$ % , and the confidence intervals are calculated by bootstrapping (or some other method that does not rely on a normal sampling distribution for the indirect effects). For these data, k = 4 IBS categories, including controls, and the adjusted confidence level is 98.3%. Because the indirect effects of each IBS subtype are all calculated with regard to the same control group, the confidence intervals are likely to be positively correlated with each other and therefore the omnibus test is likely to be conservative.

Details of primary mediation analysis and sensitivity analyses

All analyses were conducted using Stata version 11. The primary analysis used dummy variables for the IBS predictor subcategories, with healthy controls as the reference. The three subcategories were constipation-dependent, diarrhea-dependent, and alternating. The outcome variable was hourly smoothed median $\log_{10}$(cortisol), calculated for each of the six hours following the onset of stage 2 sleep. The smoothed median was calculated using a running median of span three, meaning that if three values go up, the middle one remains unchanged, but if the second value goes up and the third goes down, the middle one is replaced by the larger of the first and third values. The mediator was percent sleep period in stage 0 or 1. The first step of the two-step mediation pathway was calculated from a linear regression of the mediator on the IBS dummy variables. The total, direct and indirect effects were calculated using longitudinal generalized estimating equations (GEE) with exchangeable working correlation matrix and cubic time (hours 1-6) modeling.[3] The primary mediation mean models are:

Without the mediator, used to estimate total effect:

$$E[Y_{ij}|\mathbf{X}_i, t_j] = \beta_0 + \beta_1 X_{IBS\text{-}C} + \beta_2 X_{IBS\text{-}D} + \beta_3 X_{IBS\text{-}A} + \beta_4 t_j + \beta_5 t_j^3$$

With the mediator, used to estimate direct and indirect effects:

$$E[Y_{ij}|\mathbf{X}_i, t_j] = \beta_0 + \beta_1 X_{IBS\text{-}C} + \beta_2 X_{IBS\text{-}D} + \beta_3 X_{IBS\text{-}A} + \beta_4 X_{Mediator} + \beta_5 t_j + \beta_6 t_j^3$$

where Y_{ij} = $\log_{10}$(cortisol) of *i*th subject at time j

$\mathbf{X}_i$ = all covariates for *i*th subject

t_j = *j*th measurement time (hour); t_j^3 = *j*th hour cubed

$X_{IBS\text{-}C}$ = indicator for constipation-predominant IBS (vs. controls)

$X_{IBS\text{-}D}$ = indicator for diarrhea-predominant IBS (vs. controls)

$X_{IBS\text{-}A}$ = indicator for alternating IBS (vs. controls)

$X_{Mediator}$ = mediator value for *i*th subject

I did not include any interaction between IBS subtype and time in the model because the original analysis in the study from which these data were taken found no such interaction.

The indirect effect was calculated using the “difference of coefficients” method as the difference between the total and direct effects. Bias-corrected (BC) 95% confidence intervals for each of the total, direct and indirect effects were obtained by bootstrapping, using 10,000 repetitions.[4]

Sensitivity analyses were conducted using the same models and bootstrapping method to assess how results changed when calculating the indirect effect using the “product of coefficients” method; when using a different mediator (per-hour sleep fragmentation index); when modeling time as a linear function only; and when modeling the categorical predictor as a binary indicator for IBS (compared to controls) or a binary indicator for constipation-predominant IBS (compared to diarrhea-predominant IBS). I also conducted a sensitivity analysis to compare results obtained using GEE to results obtained using a random intercepts mixed model with an independent covariance matrix to estimate c and c'.[3]

The last set of sensitivity analyses compared the indirect effects obtained using the primary analysis method to those obtained with other mediation methods that are limited to continuous or binary predictors. For each method, I did separate analyses for each subtype vs. controls.

First, I compared the indirect effects from the primary analysis to those obtained using a mediation method (Stata command ‘sgmediation’) that only works for one-level, ‘collapsed’ data.[5–8] This method involved three OLS linear regression models: one regressing a summary outcome on a binary indicator of IBS subtype vs. controls to estimate the total effect, c; one regressing the mediator on the binary subtype indicator to estimate a; and one regressing a summary outcome on the binary subtype indicator and the mediator to estimate b and c'. The indirect effect was calculated as the product of coefficients $a*b$. I repeated the analysis with three different summary outcomes, per-subject mean, minimum, and maximum $\log_{10}$(cortisol). The mean models are:

$$E[Y] = \beta_0 + \beta_1 X_{\text{Subtype}}$$

$E[M] = \beta_0 + \beta_1 X_{Subtype}$

$E[Y] = \beta_0 + \beta_1 X_{Subtype} + \beta_2 X_{Mediator}$

where Y = per-subject mean, minimum, or maximum $\log_{10}$(cortisol)

$X_{Subtype}$ = indicator for IBS-C, IBS-D, or IBS-A (vs. controls)

$X_{Mediator}$ = percent sleep period in stage 0 or 1

Next, I calculated the indirect effects using a method designed to handle multilevel data (Stata command ‘ml_mediation’).[5] This method involved the same series of three regressions to calculate *c*, *a*, *b*, and *c′*, and also calculated the indirect effect as the product of coefficients *a***b*. However, the first and third regression models were random intercept mixed models with an independent covariance matrix, rather than OLS, and included linear and cubic time variables as covariates. The mean models are

$E[Y_{ij}] = \beta_0 + \beta_1 X_{Subtype} + \beta_2 t_j + \beta_3 t_j^3$

$E[M] = \beta_0 + \beta_1 X_{Subtype}$

$E[Y] = \beta_0 + \beta_1 X_{Subtype} + \beta_2 X_{Mediator} + \beta_3 t_j + \beta_4 t_j^3$

where $Y_{ij} = \log_{10}$(cortisol) of *i*th subject at time *j*

Finally, I calculated the indirect effects using a “seemingly unrelated estimation” cross-model comparison method that included two OLS linear regressions of $\log_{10}$(cortisol) on IBS subtype indicators and cubic time, one with and one without the mediator, and combined the parameter estimates and covariance matrices from the models with and without the mediator into a single parameter vector and robust covariance matrix; the indirect effect estimate and confidence interval were calculated as a linear combination of parameter estimates by the ‘difference of coefficients’ method.[9] The ‘suest’ command used to carry out the seemingly unrelated estimation analysis in Stata does not support panel regression models such as GEE or random-intercept mixed models, but does handle multilevel data with per-subject clustering at the level of the combined covariance matrix. The mean models used to estimate the total, direct and indirect effects were the same as the primary mediation models with the exception of being restricted to one subtype:

$$E[Y_{ij}] = \beta_0 + \beta_1 X_{Subtype} + \beta_2 t_j + \beta_3 t_j^3$$

$$E[Y_{ij}] = \beta_0 + \beta_1 X_{Subtype} + \beta_2 X_{Mediator} + \beta_3 t_j + \beta_4 t_j^3$$

Exploratory analysis of additional covariates and indirect effects

In order to assess whether indirect effects of sleep disruption as a mediator might be due to some other latent variable, I compared the indirect effects obtained using the primary mediation analysis method to those obtained when additional covariates were added to the primary mediation GEE model used to estimated direct and indirect effects, along with the mediator. No covariates were added to the no-mediator model used to estimate the total effect. The covariates were all subject-level variables and included age in years; body mass index (BMI); a measure of overall psychological distress, the global severity index (GSI) calculated from the Symptom Checklist -90-R; a measure of self-reported sleep problems during the previous month, the

Pittsburgh sleep quality index global score (PSQI); and a self-reported summary of daily stress. Adjusted mean models are:

$$\text{Age, BMI: } E[Y_{ij}|\mathbf{X}_i, t_j] = \beta_0 + \beta_1 X_{\text{IBS-C}} + \beta_2 X_{\text{IBS-D}} + \beta_3 X_{\text{IBS-A}} + \beta_4 X_{\text{Mediator}} + \beta_5 X_{\text{Age}} + \beta_6 X_{\text{BMI}} + \beta_7 t_j + \beta_8 t_j^3$$

$$\text{GSI: } E[Y_{ij}|\mathbf{X}_i, t_j] = \beta_0 + \beta_1 X_{\text{IBS-C}} + \beta_2 X_{\text{IBS-D}} + \beta_3 X_{\text{IBS-A}} + \beta_4 X_{\text{Mediator}} + \beta_5 X_{\text{GSI}} + \beta_6 t_j + \beta_7 t_j^3$$

$$\text{PSQI: } E[Y_{ij}|\mathbf{X}_i, t_j] = \beta_0 + \beta_1 X_{\text{IBS-C}} + \beta_2 X_{\text{IBS-D}} + \beta_3 X_{\text{IBS-A}} + \beta_4 X_{\text{Mediator}} + \beta_5 X_{\text{PSQI}} + \beta_6 t_j + \beta_7 t_j^3$$

$$\text{Stress: } E[Y_{ij}|\mathbf{X}_i, t_j] = \beta_0 + \beta_1 X_{\text{IBS-C}} + \beta_2 X_{\text{IBS-D}} + \beta_3 X_{\text{IBS-A}} + \beta_4 X_{\text{Mediator}} + \beta_5 X_{\text{Stress}} + \beta_6 t_j + \beta_7 t_j^3$$

Relationship of individual awakenings to changes in cortisol levels

Details of the sleep assessment and cortisol collection are reported in Burr et al.[10] For purposes of this analyis, sleep stage was available in 30 second epochs, and plasma cortisol levels were recorded at approximately 20 minute intervals, beginning before the onset of sleep and continuing until subjects awakened spontaneously or were awakened in the morning. Sleep epochs were labeled by the time at the beginning of the epoch and cortisol observations were labeled by the time at the beginning of the blood draw. A study nurse made notes of any sleep disruptions or blood draw difficulties throughout the night. Using time and study ID, I merged the sleep and cortisol data to create a combined file with sleep epoch count, sleep stage, and plasma cortisol level (ug/L), together with indicators for awakenings noted by the study nurse at the time of sampling. I denoted the awakenings as ‘major’ if the nurse noted that the patient complained of pain or discomfort or was awake for a prolonged period, ‘minor’ if the nurse noted that the awakening was brief, or ‘unsure’ if there was no descriptive information. I also added a new variable counting sleep epochs from the onset of stage 2 sleep.

For this exploratory analysis, my goal was to see if individual cortisol measurements tended to increase or decrease after awakenings. To quantify awakenings, I counted the number of awake (stage 0) epochs during the 15 minutes ending 5 minutes prior to each cortisol measurement, i.e., the 15 minutes immediately after the prior cortisol measurement. This variable had a minimum of 0, if no awakenings occurred during the 15 minute window, and a maximum of 30, if every 30-second epoch during the 15 minutes was awake. Because some subjects awakened for good in the morning before the final cortisol measurements, I dropped any observations taken more than 5 minutes after the start of the terminal awake period of the morning, in order to restrict the analysis to arousals during the sleep period. I calculated the *immediate change* in cortisol as the difference between each measurement and the prior measurement ($t_0 – t_{-1}$), and the *delayed change* as the difference between the following and current measurements ($t_{+1} – t_0$). To evaluate the relationship between prior awakenings and cortisol change, I plotted both immediate change and delayed change versus the count of awake epochs in the 15 minutes ending 5 minutes before the t_0 cortisol measurement. For both immediate and delayed change, I then ran a regression to quantify the first-order trend of the relationship, using an OLS linear regression model with clustered sandwich variance estimators and including number of sleep epochs after entering stage 2 sleep as a covariate. The mean model was:

$$E[Y] = \beta_0 + X_{\mathrm{AwakeCt}} + X_{\mathrm{SleepEpochs}}$$

where Y = cortisol change ($t_0 – t_{-1}$ or $t_{+1} – t_0$)

$X_{AwakeCt}$ = number of awake (stage 0) epochs during the 15 minutes ending 5 minutes prior to each cortisol measurement

$X_{SleepEpochs}$ = time, measured as number of sleep epochs after entering stage 2 sleep

To see if awakenings that were severe enough to be noted by the study nurse had a greater or more consistent effect than awakenings in general, I repeated the plots and regressions after restricting the analysis to samples where the nurse noted an awakening at t_0, or close to it.

Next, I explored whether the relationship between cortisol change and prior awakenings was different during early, middle and late sleep by repeating the plots for immediate and delayed change, separating the first 2 hours of sleep, the middle 3 hours, and the final 3 hours. Finally, I used additional plots to explore whether there was any effect modification from covariates including IBS subtype, age, BMI, and a measure of psychological distress, GSI.

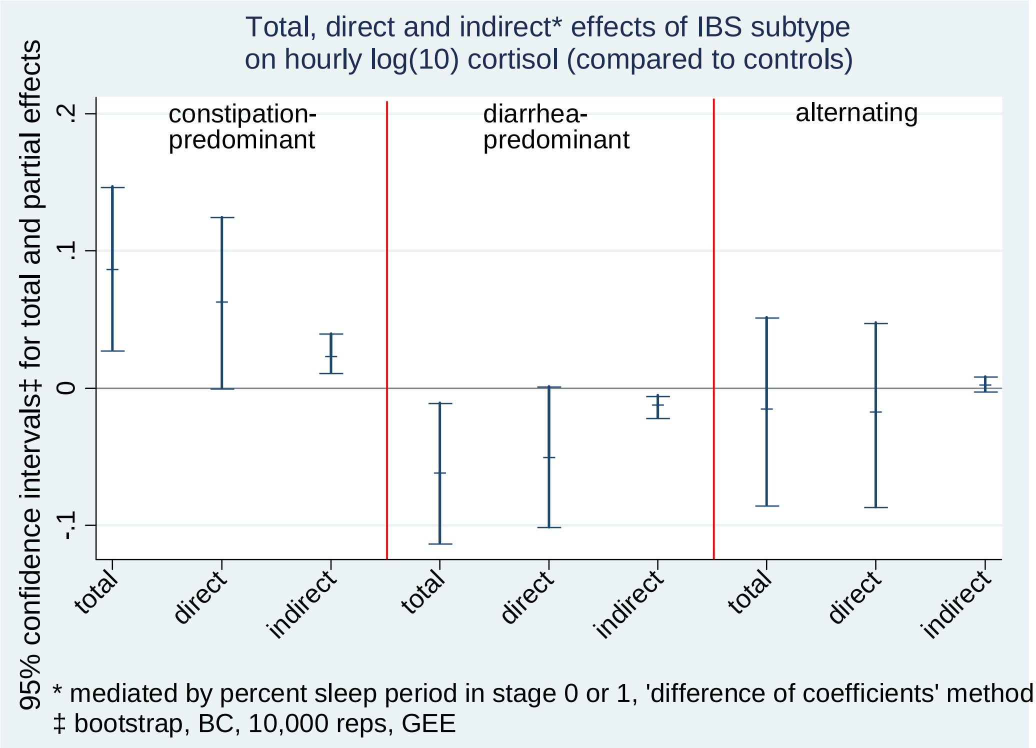
For the last part of this exploratory analysis, I looked at whether awakenings tended to be clustered before the steepest cortisol increases or after a local minimum, and whether fewer awakenings tended to be found before cortisol decreases. To do this, I created indicators for immediate change of 5 ug/L or more (the top 5% of change values) and decreasing cortisol (immediate change < 0, the lower 50% of change values). I also created indicators for being a local minimum, i.e., a cortisol value lower than both the previous and following values, and following a local minimum. I then divided the count of awake epochs during the 15 minutes ending 5 minutes prior to each cortisol measurement into categories (0 epochs, 1-15 epochs, 16-29 epochs, and 30 epochs) and cross-tabulated the categories with the cortisol change indicators.

All analyses were conducted using Stata version 11.2 (StataCorp LP, College Station, TX).

Results

Primary mediation analysis

The primary mediation analysis using dummy variables for IBS subtypes did find evidence to support the existence of indirect effects (Figure 1).



Total, direct and indirect* effects of IBS subtype on hourly log(10) cortisol (compared to controls)

* mediated by percent sleep period in stage 0 or 1, 'difference of coefficients' method
‡ bootstrap, BC, 10,000 reps, GEE

Figure 1. 95% confidence intervals for total, direct and indirect effects of IBS subtype on nocturnal cortisol levels, mediated by percent sleep period in stage 0 or 1.

	Ratio of Geometric Means, IBS Subtype vs. Controls (95% CI)		
IBS Subtype	Total Effect	Direct Effect	Indirect Effect
Constipation-predominant	1.22 (1.07, 1.40)	1.16 (1.00, 1.33)	1.06 (1.03, 1.10)
Diarrhea-predominant	0.87 (0.77, 0.98)	0.89 (0.79, 1.00)	0.97 (0.95, 0.99)
Alternating	0.97 (0.82, 1.13)	0.96 (0.82, 1.12)	1.01 (1.00, 1.02)

The direction and magnitude of the total effects are consistent with those found in the original study by Burr et al. from which these data were taken, who found that compared to controls, mean plasma cortisol levels were higher in constipation-predominant subjects and lower in diarrhea-predominant subjects, while alternating subjects had mean levels very close to controls[1]. For both constipation-predominant and diarrhea-predominant subjects, the estimated indirect effect of IBS subtype mediated through sleep disruption is small, but not trivial as a proportion of the estimated total effect, equaling over 20% of the total effect. The indirect effect of IBS subtype mediated through sleep disruption is positive for constipation-predominant subjects – i.e., tending to increase mean plasma cortisol relative to controls – and negative for diarrhea-predominant subjects, i.e., tending to decrease mean plasma cortisol relative to controls. This is consistent with a trend for more disturbed sleep to result in higher nocturnal cortisol, since constipation-predominant subjects tend to have higher percent sleep period in stage 0 or 1 compared to controls and diarrhea-predominant subjects tend to have lower percent sleep period in stage 0 or 1 compared to controls (Figure 2). Note that a single IBS-diarrhea subject has a high outlier value for sleep disruption (arrow in Figure 2). In this small sample, this single subject has a substantial influence on the indirect effect estimate, as shown in Figure 3. Omitting this subject from the analysis results in a larger (more negative) estimate for the indirect effect of IBS-diarrhea mediated through sleep disruption, compared to controls.

One additional point of interest in Figures 1 and 3 is that the direct and indirect effects are both positive for constipation-predominant subjects and both negative for diarrhea-predominant subjects. Both indirect and direct effects can be considered as partial effects, carrying some of the influence of the predictor to the outcome. The partial effects need not reinforce each other; it is possible for them to act in opposite directions, one tending to increase and one tending to decrease the response variable. It appears that the partial effect of sleep disruption, together with some other aspect (or aspects) of IBS summarized in the direct effect estimate, may tend to work together to increase nocturnal cortisol in constipation-predominant subjects and decrease nocturnal cortisol in diarrhea-predominant subjects, relative to controls.

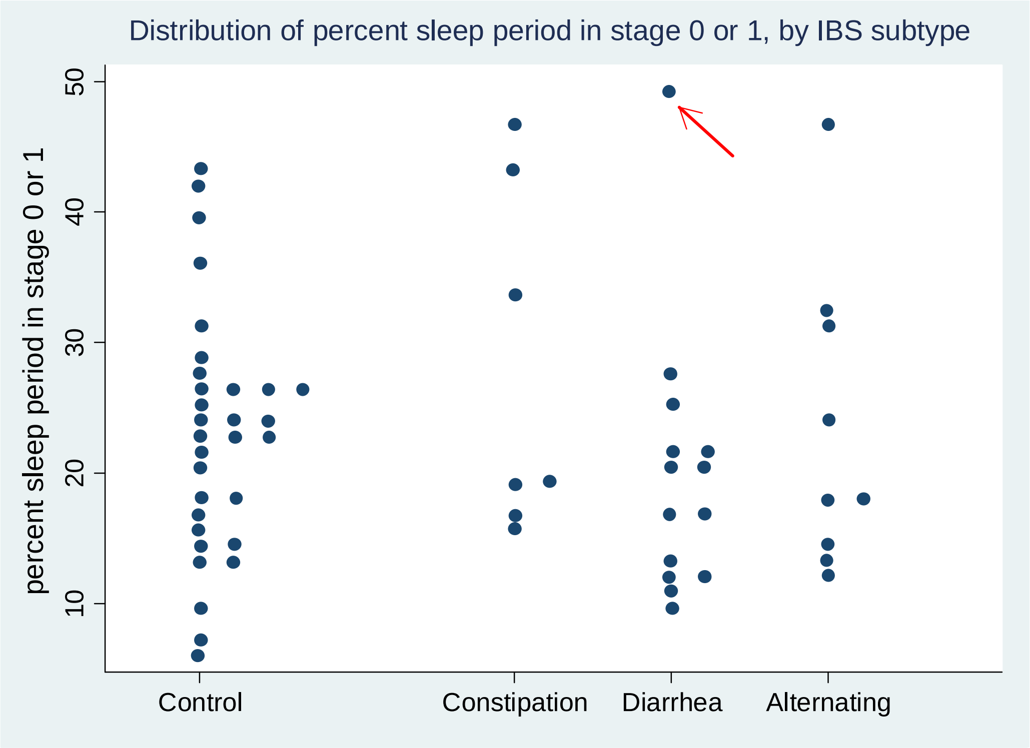


Figure 2. Distribution of percent sleep period in stage 0 or 1 across IBS subtypes. Compared to controls, the mean is 5.0 percentage points higher for constipation-predominant subjects and 2.5 percentage points lower for diarrhea-predominant subjects.

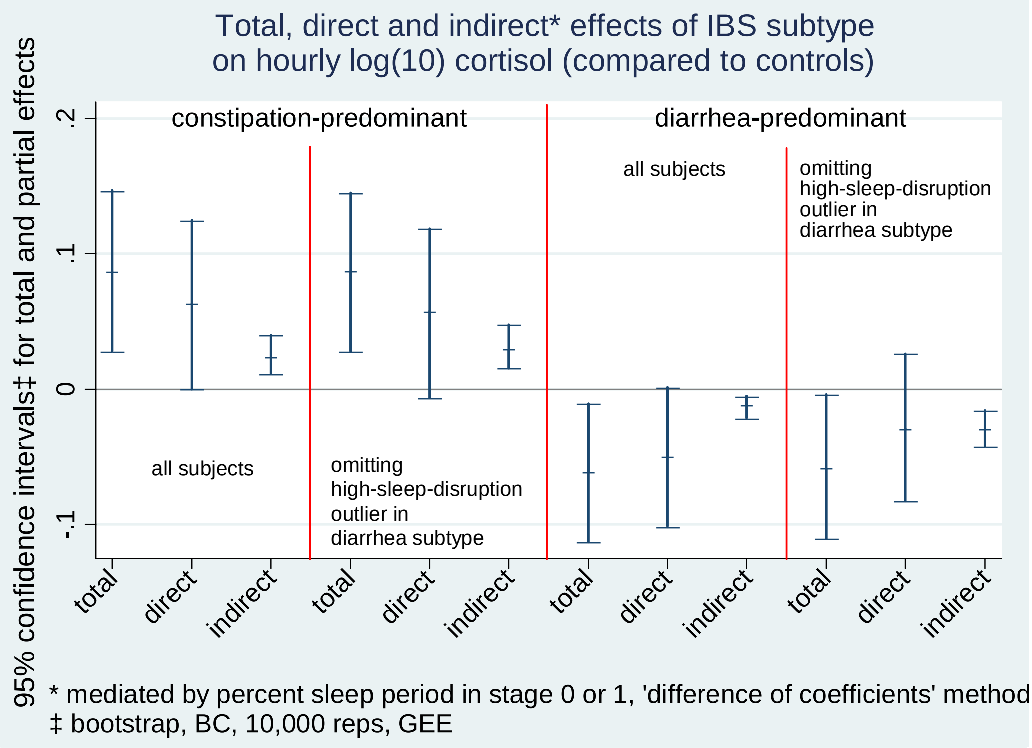


Figure 3. Omitting the IBS-diarrhea subject with a high outlier value for sleep disruption results in a larger indirect effect estimate for IBS effect on cortisol mediated by sleep disruption.

	Ratio of Geometric Means, IBS Subtype vs. Controls (95% CI)		
IBS Subtype	Total Effect	Direct Effect	Indirect Effect
Constipation-predominant, all subjects	1.22 (1.07, 1.40)	1.16 (1.00, 1.33)	1.06 (1.03, 1.10)
Constipation-predominant, omitting outlier	1.22 (1.07, 1.40)	1.14 (0.99, 1.31)	1.07 (1.04, 1.12)
Diarrhea-predominant, all subjects	0.87 (0.77, 0.98)	0.89 (0.79, 1.00)	0.97 (0.95, 0.99)
Diarrhea-predominant, omitting outlier	0.87 (0.78, 0.99)	0.93 (0.83, 1.06)	0.94 (0.91, 0.96)

Inference for mediation analysis

Figure 4 shows confidence intervals that were Bonferroni adjusted for 3 comparisons to test the null hypothesis that all the indirect effects equal zero.

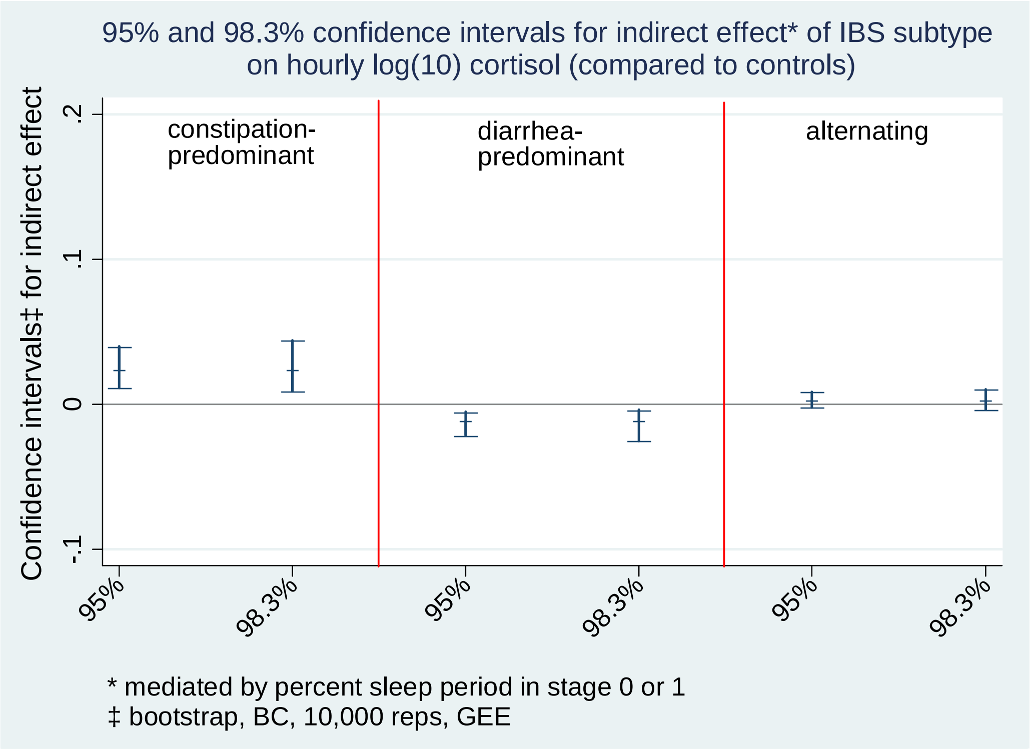


Figure 4. 95% and 98.3% confidence intervals for indirect effects of IBS subtype on nocturnal cortisol levels, mediated by percent sleep period in stage 0 or 1.

	Ratio of Geometric Means, IBS Subtype vs. Controls	
IBS Subtype	Indirect Effect, 95% CI	Indirect Effect, 98.3% CI
Constipation-predominant	(1.026, 1.096)	(1.021, 1.107)
Diarrhea-predominant	(0.952, 0.988)	(0.944, 0.991)
Alternating	(0.995, 1.020)	(0.991, 1.024)

The 98.3% confidence intervals exclude zero for both the constipation-predominant and diarrhea-predominant indirect effect estimates. Because they compare to a common reference category, the estimate and confidence intervals are likely to be positively correlated, which would make this a conservative test. This is evidence in favor of rejecting the null hypothesis and concluding that the indirect effects of IBS on nocturnal cortisol mediated through sleep disruption may indeed be real, nonzero effects for one or more IBS subtypes.

Sensitivity analyses

The first sensitivity analysis compared indirect effects calculated using the ‘product of coefficients’ and ‘difference of coefficients’ methods (Figure 5).

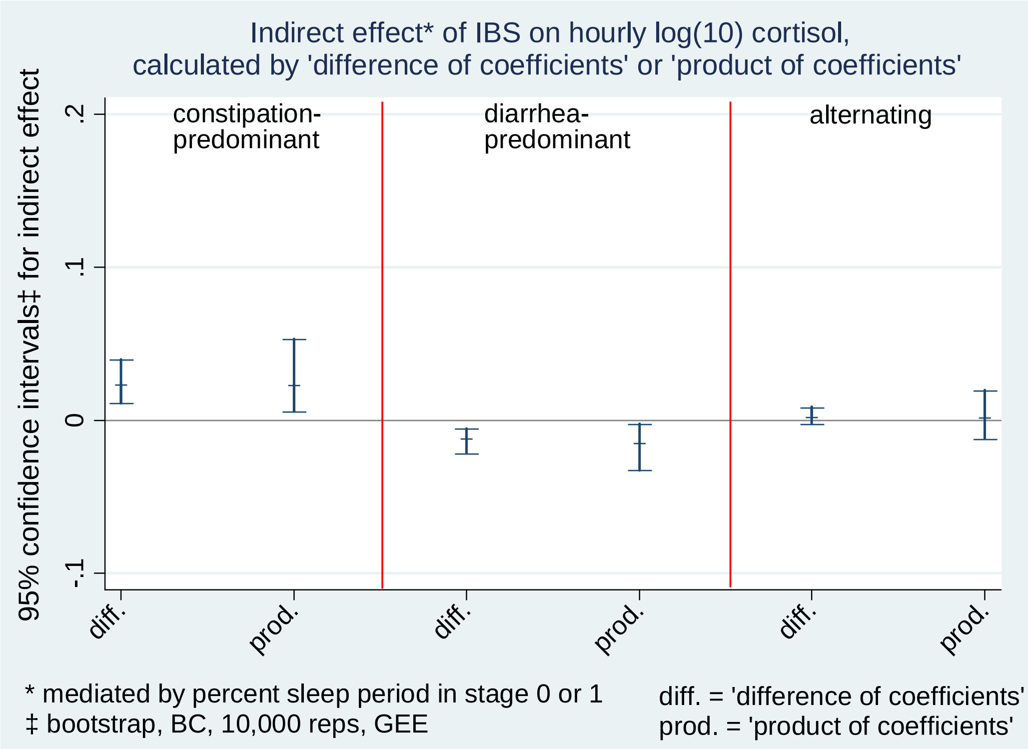


Figure 5. Confidence intervals for indirect effects of IBS subtype on nocturnal cortisol levels, calculated using the ‘difference of coefficients’ or ‘product of coefficients’ method.

	Ratio of Geometric Means, IBS Subtype vs. Controls (95% CI)	
IBS Subtype	Difference Method ($c_i - c'_i$)	Product Method ($a_i * b$)
Constipation-predominant	1.06 (1.03, 1.10)	1.06 (1.01, 1.13)
Diarrhea-predominant	0.97 (0.95, 0.99)	0.97 (0.93, 0.99)
Alternating	1.01 (1.00, 1.02)	1.01 (0.97, 1.05)

The point estimates for indirect effects were the same to two decimal places, but using the ‘product of coefficients’ method resulted in wider confidence intervals. It appears that the conceptual advantage of the difference method for multilevel data, where the indirect effect is calculated from two coefficients that both come from multilevel models ($c_i - c'_i$), rather than from one coefficient from a single-level model and one coefficient from a multilevel model (a_i*b), may be matched by a practical advantage of greater efficiency.

Figure 6 shows indirect effects mediated through two different summaries of sleep disruption, percent sleep period in stage 0 or 1 vs. a per-hour fragmentation index.

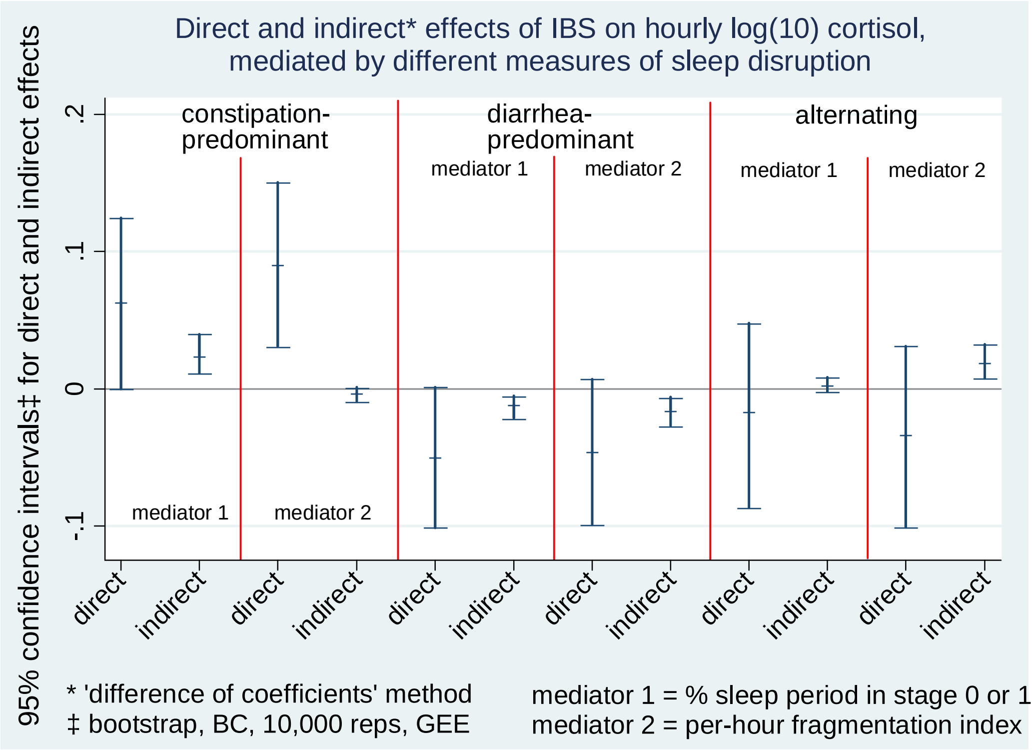


Figure 6. 95% confidence intervals for direct and indirect effects of IBS on log(10) cortisol, mediated by two different measures of sleep disruption.

	Ratio of Geometric Means, IBS Subtype vs. Controls (95% CI)			
	Percent sleep period in stage 0 or 1		Per-hour sleep fragmentation index	
IBS Subtype	Direct Effect	Indirect Effect	Direct Effect	Indirect Effect
Constipation-predominant	1.16 (1.00, 1.33)	1.06 (1.03, 1.10)	1.23 (1.07, 1.41)	0.99 (0.98, 1.00)
Diarrhea-predominant	0.89 (0.79, 1.00)	0.97 (0.95, 0.99)	0.90 (0.80, 1.02)	0.96 (0.94, 0.99)
Alternating	0.96 (0.82, 1.12)	1.01 (1.00, 1.02)	0.93 (0.79, 1.07)	1.05 (1.02, 1.08)

For diarrhea-predominant subjects, indirect effects are similar for both mediators. For constipation-predominant subjects, the indirect effect mediated by the per-hour fragmentation index is opposite in sign, as well as smaller and less significant, compared to the indirect effect mediated by percent sleep period in stage 0 or 1. For alternating subjects, the indirect effect mediated by the per-hour fragmentation index is larger and more significant. These differences may be the result of random variation or multiple comparisons, but looking across IBS subtypes and mediators, the data are consistent with IBS exerting some influence on nocturnal cortisol mediated by sleep disruption, which may possibly vary by IBS subtype and measure of sleep disruption.

Interestingly, for alternating subjects, indirect effect estimates of both mediators are positive while direct effect estimates are negative, hinting at a possibility that the nonsignificant, near-zero total effect estimate seen in Figure 1 may be partly due to conflicting direct and indirect effects.

The primary analysis modeled time as a cubic variable. When $\log_{10}$(cortisol) is regressed against time (hours since entering stage 2 sleep) and time cubed in a GEE model with per-subject clustering, the Wald test for the addition of the cubic time variable is statistically significant ($p = 0.045$). This confirms the nonlinearity apparent in individual plots of $\log_{10}$(cortisol) vs. time (Figure 7) and a combined plot of $\log_{10}$(cortisol) vs. time for all subjects (Figure 8).

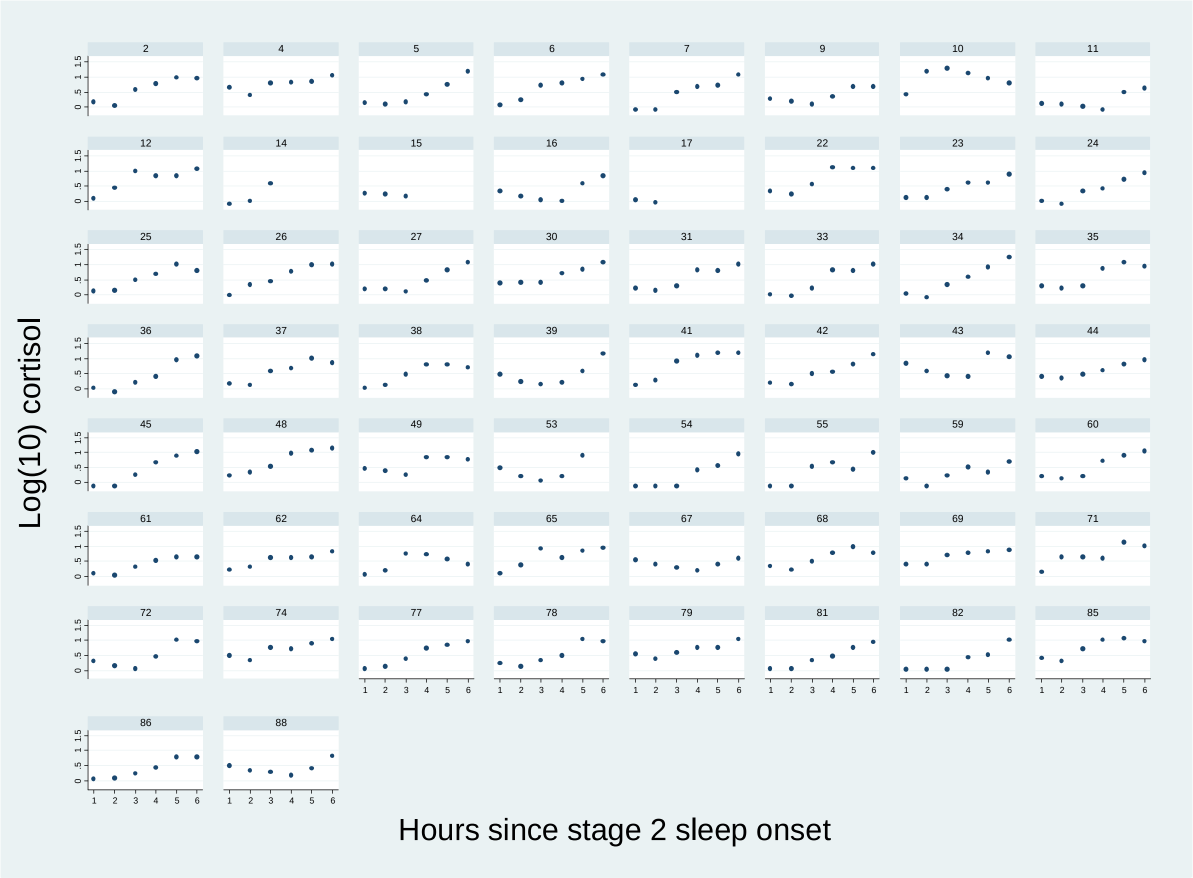


Figure 7. Individual trajectories of log(10) cortisol vs. hours since onset of stage 2 sleep.

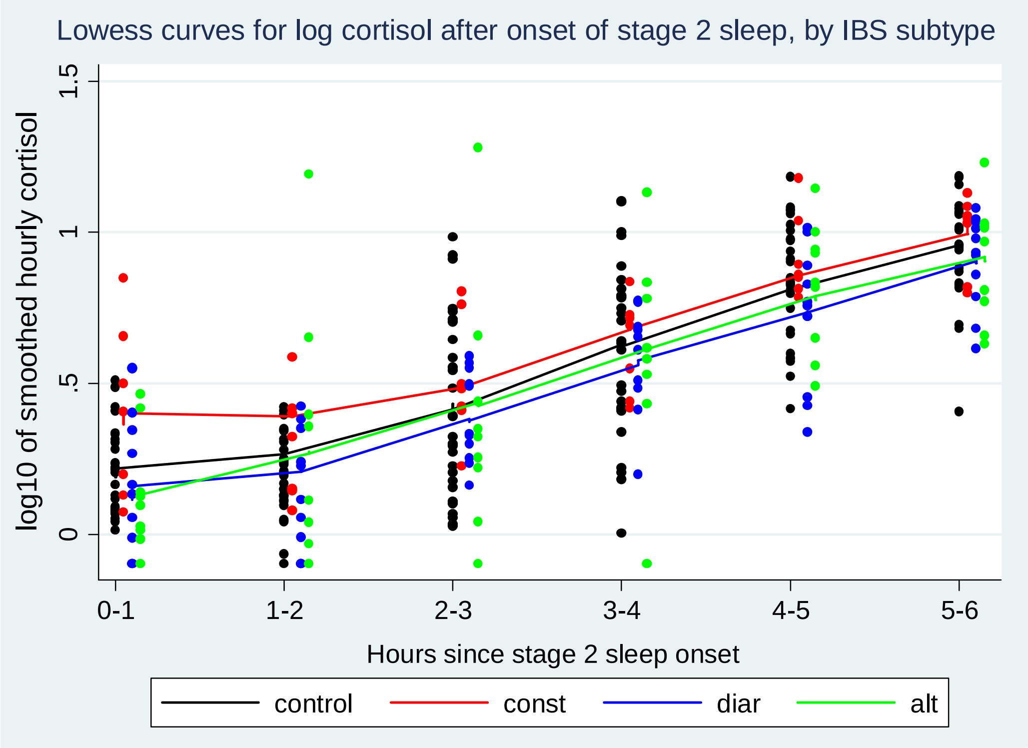


Figure 8. Lowess curves of log(10) cortisol vs. hours since onset of stage 2 sleep, by IBS subtype.

However, a comparison of the fitted values from the linear and cubic time models shows that they are quite close (Figure 9). Because of this, I ran a sensitivity analysis to see if omitting the cubic time variable would change the results of the main mediation analysis, and found that the total, direct and indirect effect estimates were essentially unchanged (Figure 10).

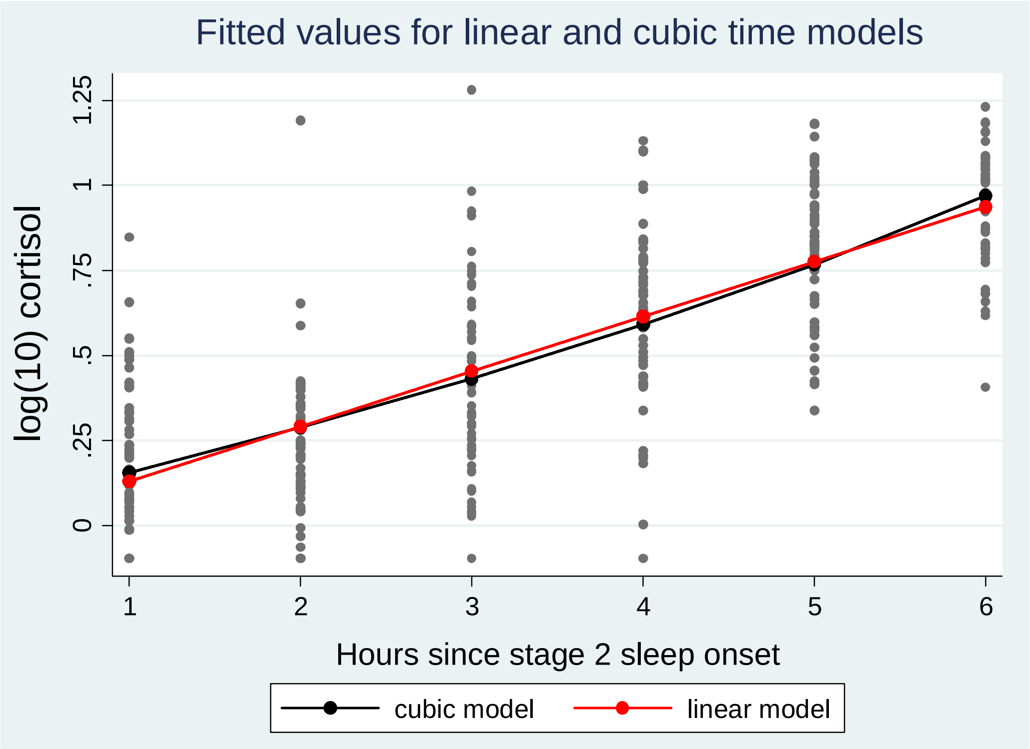


Figure 9. Fitted values from GEE models regressing log(10) cortisol on time (hours since onset of stage 2 sleep), with and without a cubic time variable.

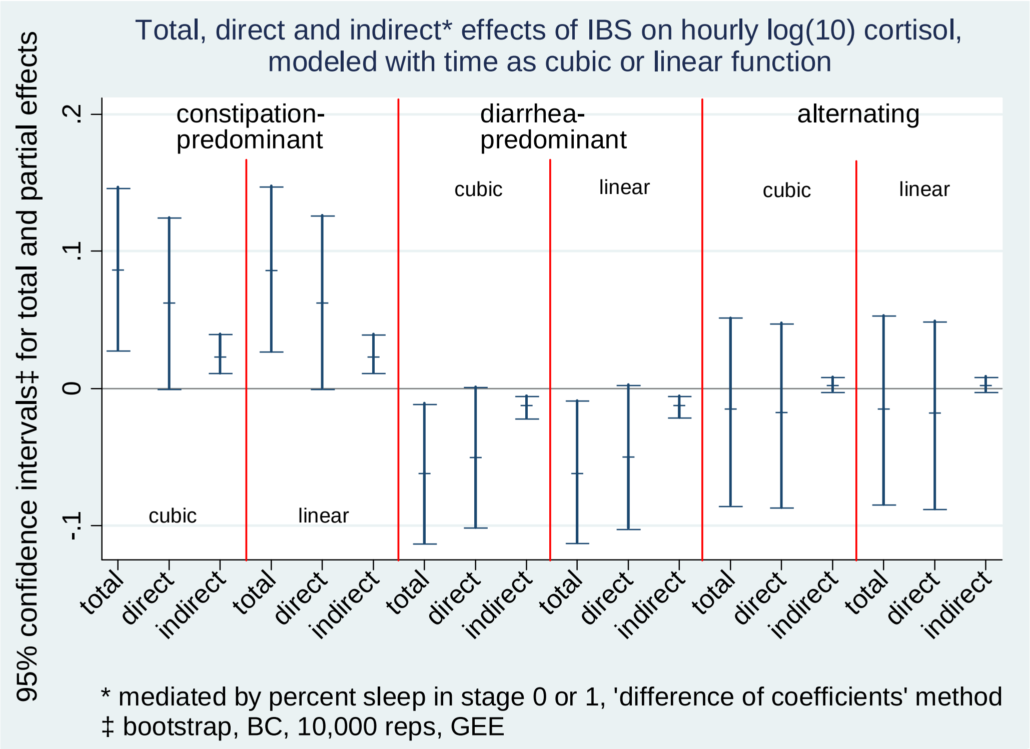


Figure 10. Comparison of mediation effect estimates when modeling time as a cubic or linear variable.

	Ratio of Geometric Means, IBS Subtype vs. Controls (95% CI)		
IBS Subtype	Total Effect	Direct Effect	Indirect Effect
Constipation-predominant, cubic time	1.22 (1.07, 1.40)	1.16 (1.00, 1.33)	1.06 (1.03, 1.10)
Constipation-predominant, linear time	1.22 (1.06, 1.41)	1.16 (1.00, 1.34)	1.06 (1.03, 1.10)
Diarrhea-predominant, cubic time	0.87 (0.77, 0.98)	0.89 (0.79, 1.00)	0.97 (0.95, 0.99)
Diarrhea-predominant, linear time	0.87 (0.77, 0.98)	0.89 (0.79, 1.01)	0.97 (0.95, 0.99)
Alternating, cubic time	0.97 (0.82, 1.13)	0.96 (0.82, 1.12)	1.01 (1.00, 1.02)
Alternating, linear time	0.97 (0.82, 1.13)	0.96 (0.82, 1.12)	1.01 (1.00, 1.02)

The next sensitivity analysis compared mediation effect confidence intervals calculated using a GEE model with per-subject clustering and exchangeable working correlation matrix to those calculated using a mixed model with random intercepts and an independent covariance matrix. Neither model used robust (sandwich) standard errors. Both the GEE and the mixed models included a cubic variable for time. The confidence intervals were very similar for both models (Figure 11).

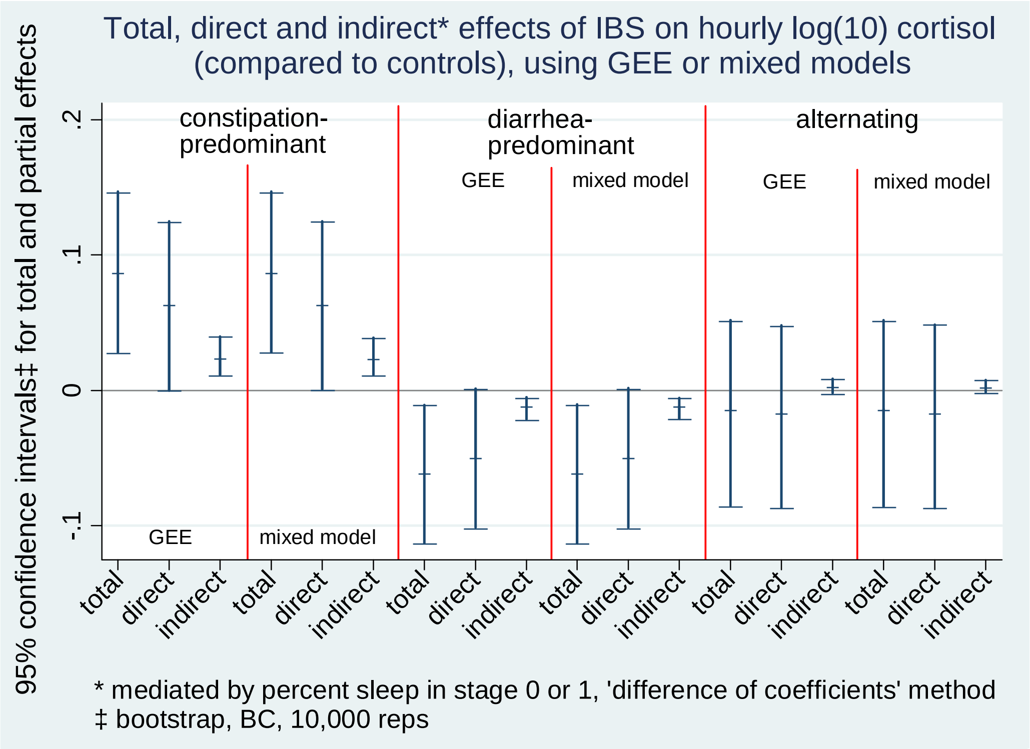
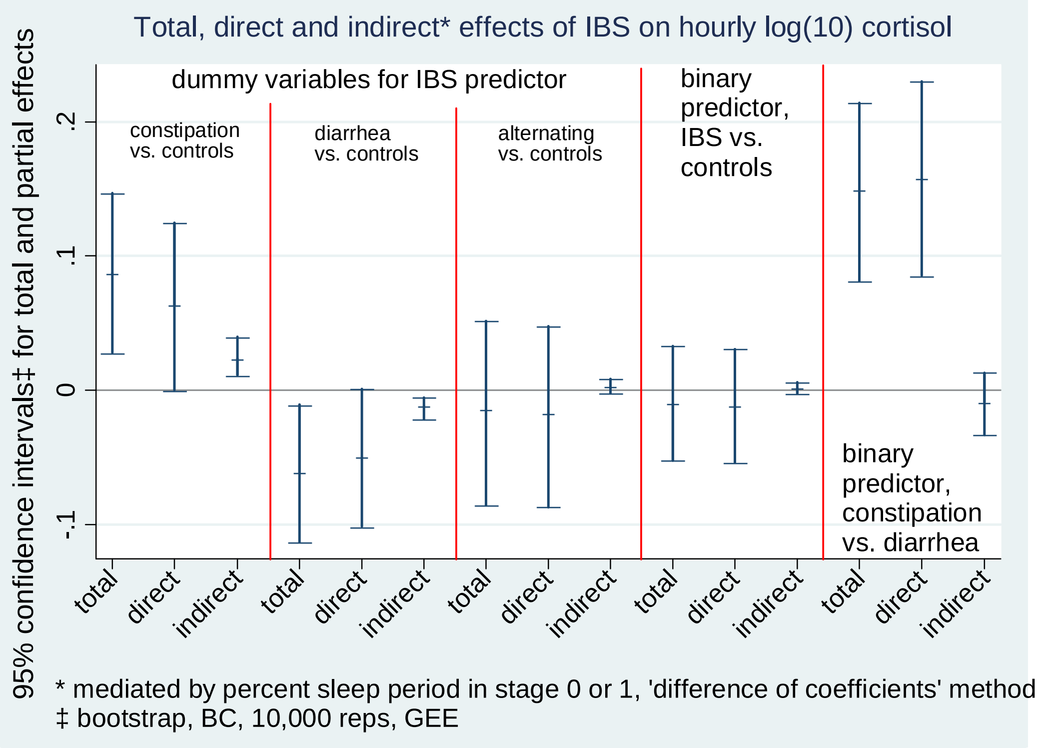


Figure 11. Confidence intervals for indirect effects of IBS subtype on nocturnal cortisol levels, calculated using GEE or a random-intercepts mixed model.

	Ratio of Geometric Means, IBS Subtype vs. Controls (95% CI)	
IBS Subtype	GEE	Mixed Model
Constipation-predominant	1.06 (1.03, 1.10)	1.06 (1.03, 1.09)
Diarrhea-predominant	0.97 (0.95, 0.99)	0.97 (0.95, 0.99)
Alternating	1.01 (1.00, 1.02)	1.01 (1.00, 1.02)

Figure 12 a) shows the total and partial effects obtained when using binary predictors, first comparing all IBS subjects to controls and then comparing constipation-predominant subjects to diarrhea-predominant subjects. Total and partial effect estimates in the IBS vs. controls comparison are close to zero, which makes sense given that different IBS subtypes (constipation-predominant and diarrhea-predominant) are associated with opposite effects on plasma cortisol levels, at least in this sample. In the absence of a mediation method developed for categorical predictors, using a binary predictor comparing disease to controls might be a reasonable choice, but would yield little information about the detailed relationships of disease subtype, mediator and outcome.

The comparison of constipation-predominant and diarrhea-predominant subjects is notable because it shows little or no indirect effect of subtype on plasma cortisol. If this result is not just due to chance, it is somewhat surprising because the two subtypes do appear to differ in percent sleep period in stage 0 or 1 (Figure 2), and the bootstrap estimate for coefficient *a*, quantifying the relationship between IBS subtype and percent sleep period in stage 0 or 1, is large and significant (*a* = 10.4, 95% CI 6.6 to 14.4). However, it appears that the second part of the mediation pathway, the association between percent sleep period in stage 0 or 1 and $\log_{10}$(cortisol), is weak in constipation-predominant and diarrhea-predominant subjects, as shown in Figure 12 b) and by the small and nonsignificant bootstrap estimate of coefficient *b* (-0.001, 95% CI -0.005 to 0.002). Chance may well be playing a large role in this result; as Figure 12 b) emphasizes, the sample size is small, particularly in the constipation and diarrhea subgroups.



Total, direct and indirect* effects of IBS on hourly log(10) cortisol

* mediated by percent sleep period in stage 0 or 1, 'difference of coefficients' method
‡ bootstrap, BC, 10,000 reps, GEE

Figure 12 a). Total, direct and indirect effects of IBS on log(10) cortisol, comparing results when IBS subtypes are compared to controls, when all IBS subjects are compared to controls, and when constipation-predominant subjects are compared to diarrhea-predominant subjects.

	Ratio of Geometric Means (95% CI)				
	Dummy variables for categorical predictor			Binary predictors	
	Constipation vs. controls	Diarrhea vs. controls	Alternating vs. controls	IBS vs. controls	Constipation vs. diarrhea
Total Effect	1.22 (1.07, 1.40)	0.87 (0.77, 0.98)	0.97 (0.82, 1.13)	0.98 (0.89, 1.08)	1.41 (1.21, 1.64)
Direct Effect	1.16 (1.00, 1.33)	0.89 (0.79, 1.00)	0.96 (0.82, 1.12)	0.97 (0.88, 1.07)	1.44 (1.22, 1.70)
Indirect Effect	1.06 (1.03, 1.10)	0.97 (0.95, 0.99)	1.01 (1.00, 1.02)	1.00 (1.00, 1.01)	0.98 (0.93, 1.03)

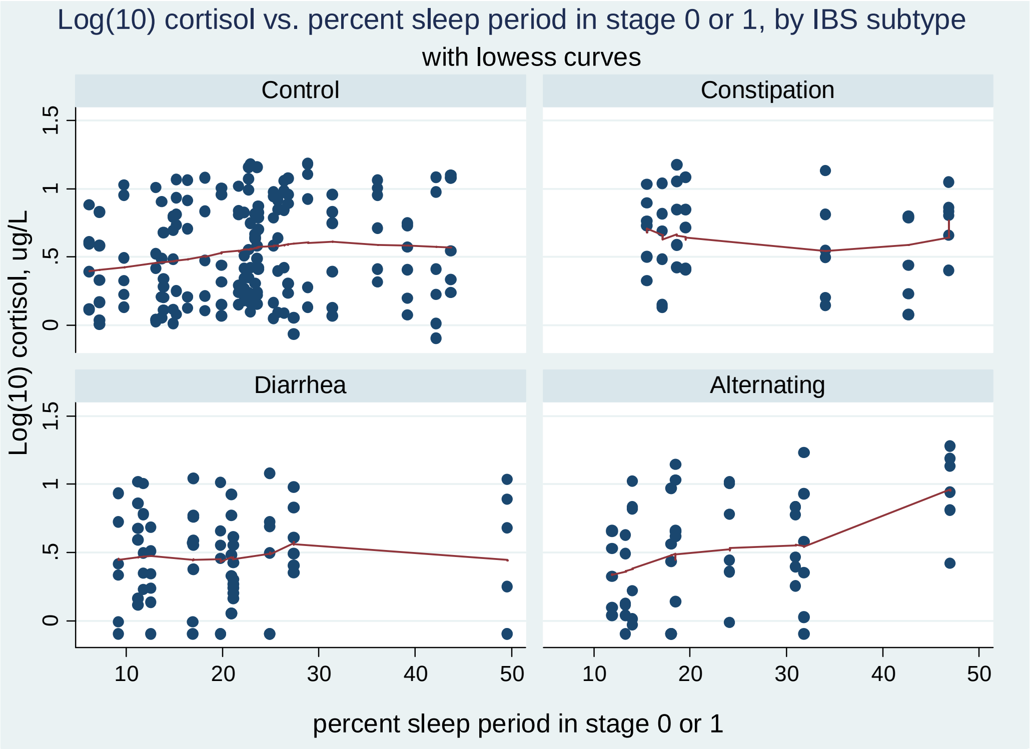


Figure 12 b). Subtype-specific relationships between mediator percent sleep period in stage 0 or 1 and outcome log(10) cortisol.

The next set of sensitivity analyses compared the results of the primary, longitudinal data analysis to results obtained using an OLS regression mediation method that only works for one-level, ‘collapsed’ data and calculates the indirect effect as the product of coefficients *a***b*, where *a* is the coefficient when mediator is regressed on IBS subtype and *b* is the coefficient when a summary outcome is regressed on the mediator (Figure 13).[2–4] The collapsed data method used per-subject mean, maximum, or minimum $\log_{10}$(cortisol) as the outcome. It did not allow dummy variables for categorical predictors, so I had to do separate analyses restricted to each subtype vs. controls. Not surprisingly, using summary outcomes and restricting the analyses to one subtype vs. controls resulted in a loss of precision, as seen in the wider confidence intervals for the collapsed data compared to the longitudinal data. Nonetheless, a consistent trend is visible even in the collapsed data: indirect effects of IBS-constipation on cortisol mediated by sleep disruption tend to be small and positive, while indirect effects of IBS-diarrhea on cortisol mediated by sleep disruption tend to be small and negative. Indirect effects of IBS-alternating on cortisol mediated by sleep disruption (not shown in plots) are all approximately zero.

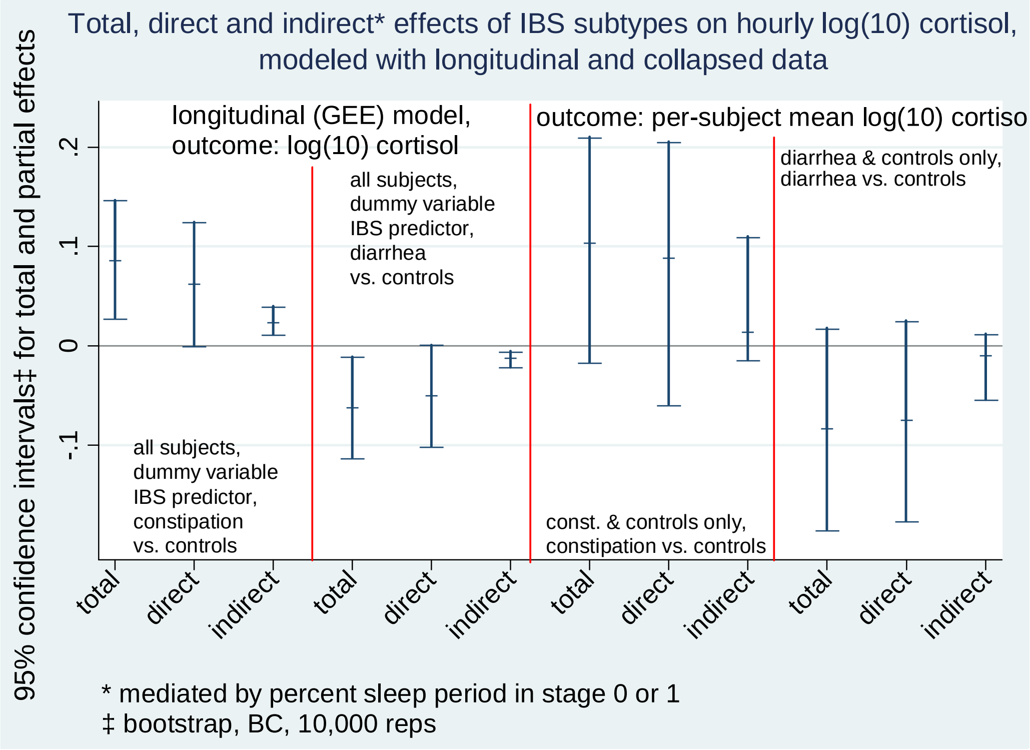


Figure 13 a). Total, direct and indirect effects of IBS subtypes on log(10) cortisol, comparing a longitudinal GEE model with a cubic time variable and dummy variables for the IBS predictor to a one-level model with per-subject mean log(10) cortisol as the outcome and a binary predictor for IBS subtype vs. controls (Stata command ‘sgmediation’).

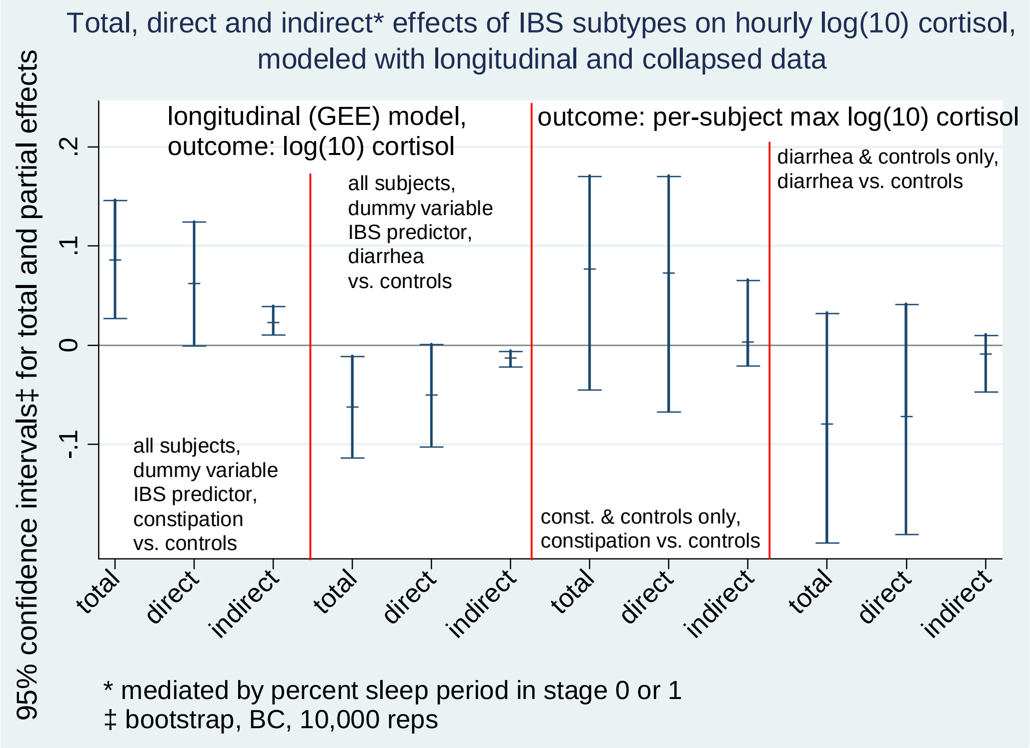


Figure 13 b). Total, direct and indirect effects of IBS subtypes on log(10) cortisol, comparing a longitudinal GEE model with dummy variables for the IBS predictor to a one-level model with per-subject maximum log(10) cortisol as the outcome and a binary predictor for IBS subtype vs. controls.

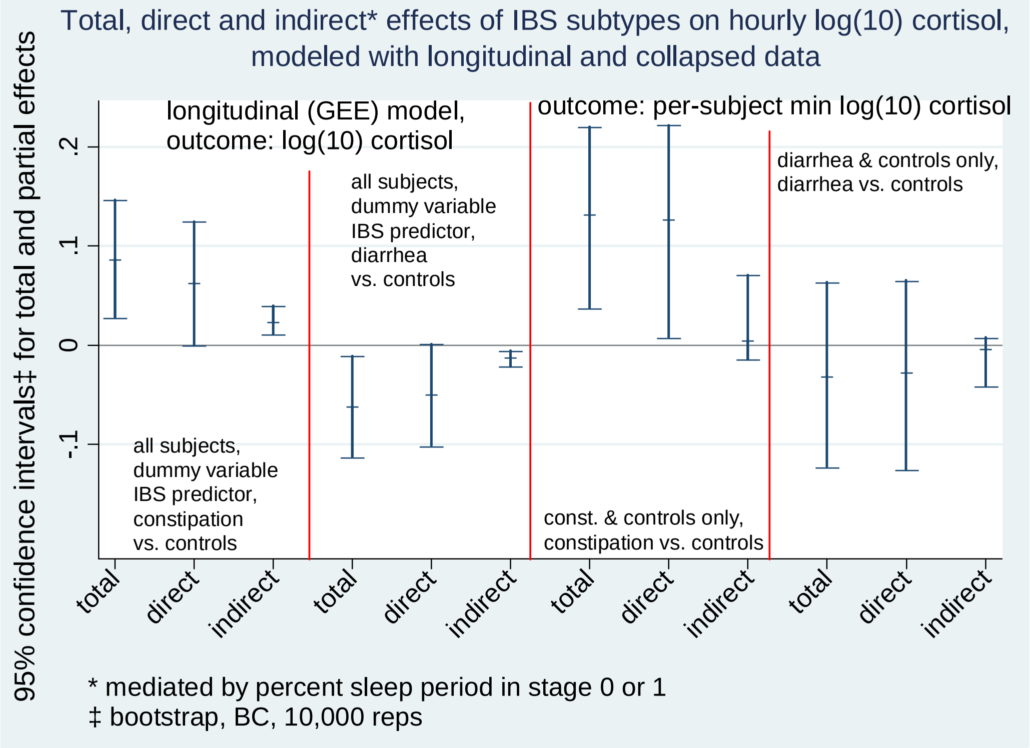


Figure 13 c). Total, direct and indirect effects of IBS subtypes on log(10) cortisol, comparing a longitudinal GEE model with dummy variables for the IBS predictor to a one-level model with per-subject minimum log(10) cortisol as the outcome and a binary predictor for IBS subtype vs. controls.

The last sensitivity analysis compared the total and partial effect estimates from the primary categorical predictor mediation method to those calculated using a different mediation method that was developed for longitudinal data, but requires a continuous or binary predictor (Figure 14).[5] Both methods included a cubic time variable, used a random effects mixed model, calculated the indirect effects as the product of coefficients, and used bootstrapped standard errors for the confidence intervals. However, ‘ml_mediation’ did not allow dummy variables for the predictor and therefore required separate analyses restricted to each subtype vs. controls. Once again, there was a trend for indirect effects of IBS-constipation on cortisol mediated by sleep disruption to be small and positive and for indirect effects of IBS-diarrhea to be small and negative. Indirect effects of IBS-alternating continued to be essentially zero (not included in plot). Finally, I did a "seemingly unrelated estimation" analysis that combined two OLS regressions of $\log_{10}$(cortisol) on IBS subtype indicators and cubic time, one with and one without the mediator percent sleep period in stage 0 or 1, into a joint covariance matrix with per-subject clustering, then calculated the indirect effect as $c - c'$, the difference between the IBS coefficients in the models with and without the mediator.[6] These results were similar to those in Figure 14.

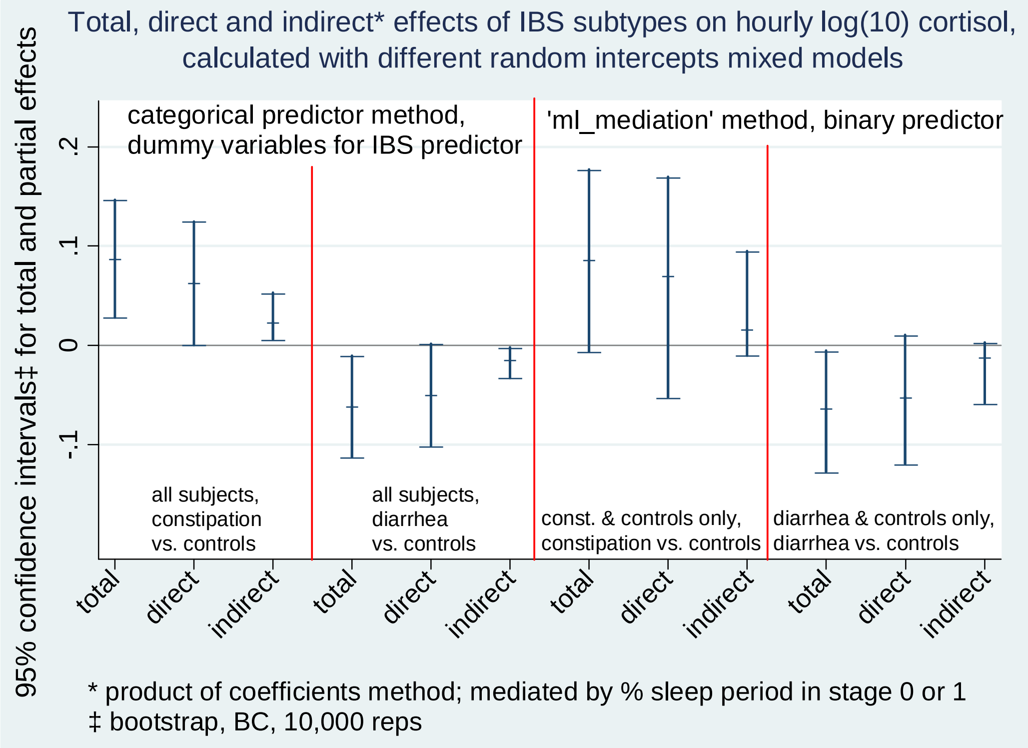


Figure 14. Total, direct and indirect effects of IBS subtypes on log(10) cortisol, comparing different longitudinal data methods. Both used cubic time variables and random intercepts mixed models, and calculated the indirect effect as a product of coefficients. The 'ml_mediation' method does not allow categorical predictors and so the analyses were restricted to one IBS subtype vs. controls.

Exploratory analysis of additional covariates and indirect effects

Figure 15 summarizes the results when additional covariates are added to the primary model estimating mediation effects. Each of the additional models controlled for one of the following list of variables: age and BMI; GSI, a measure of overall psychological distress; or PSQI, a measure of self-reported sleep problems. It appears that adjusting for age and BMI slightly increases the magnitude of indirect effects across all IBS subtypes. Results after controlling for measures of psychological distress (GSI), self-reported sleep problems (PSQI) and self-reported daily stress are less consistent; it does not appear that adjusting for any one of them causes the indirect effect of sleep disruption to disappear, or increase, in all IBS subtypes. While it is possible that there are subtype-specific effects of the covariates, it is also possible that the varying results are due to chance alone.

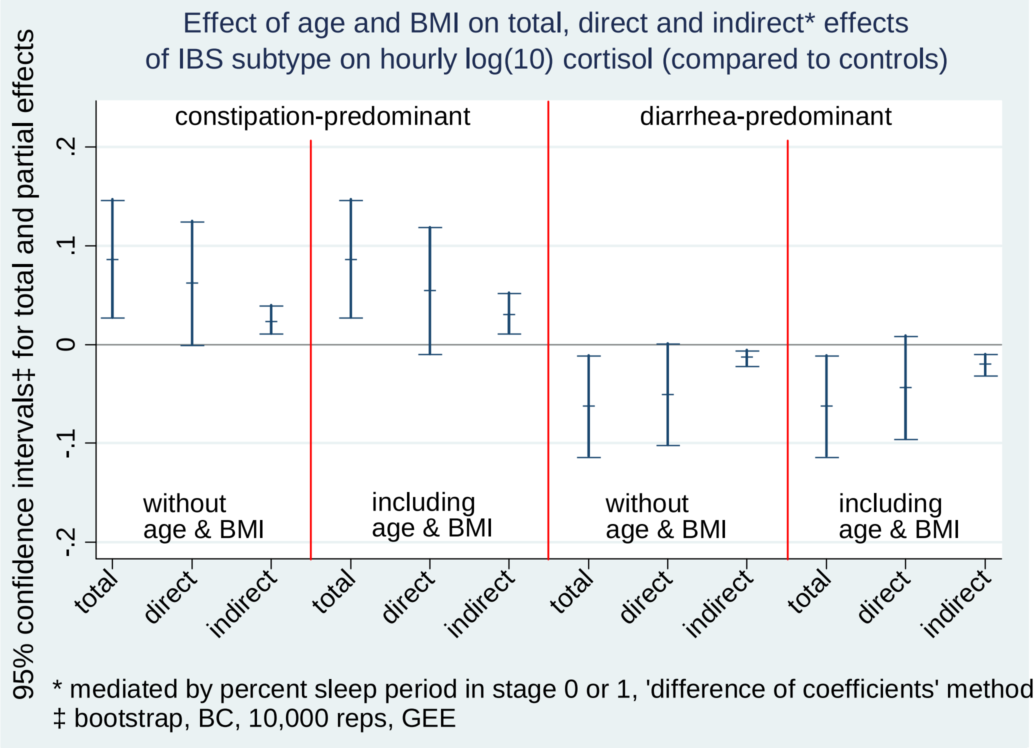


Figure 15 a). Confidence intervals for indirect effects of IBS subtype on nocturnal cortisol levels, with and without age & BMI in the mediation model. Table presents results of all models controlling for additional covariates.

	Ratio of Geometric Means, IBS Subtype vs. Controls (95% CI)				
	Additional Covariates Added to Model				
IBS Subtype	none	age & BMI	GSI	PSQI	daily stress
Constipation-predominant	1.06	1.08	1.08	0.99	1.04
	(1.03, 1.09)	(1.03, 1.13)	(1.00, 1.16)	(0.94, 1.04)	(0.97, 1.12)
Diarrhea-predominant	0.97	0.96	0.99	0.93	0.97
	(0.95, 0.99)	(0.93, 0.98)	(0.95, 1.02)	(0.89, 0.96)	(0.94, 0.99)
Alternating	1.01	1.03	1.02	0.94	0.99
	(1.00, 1.02)	(1.00, 1.06)	(0.97, 1.08)	(0.89, 0.98)	(0.95, 1.04)

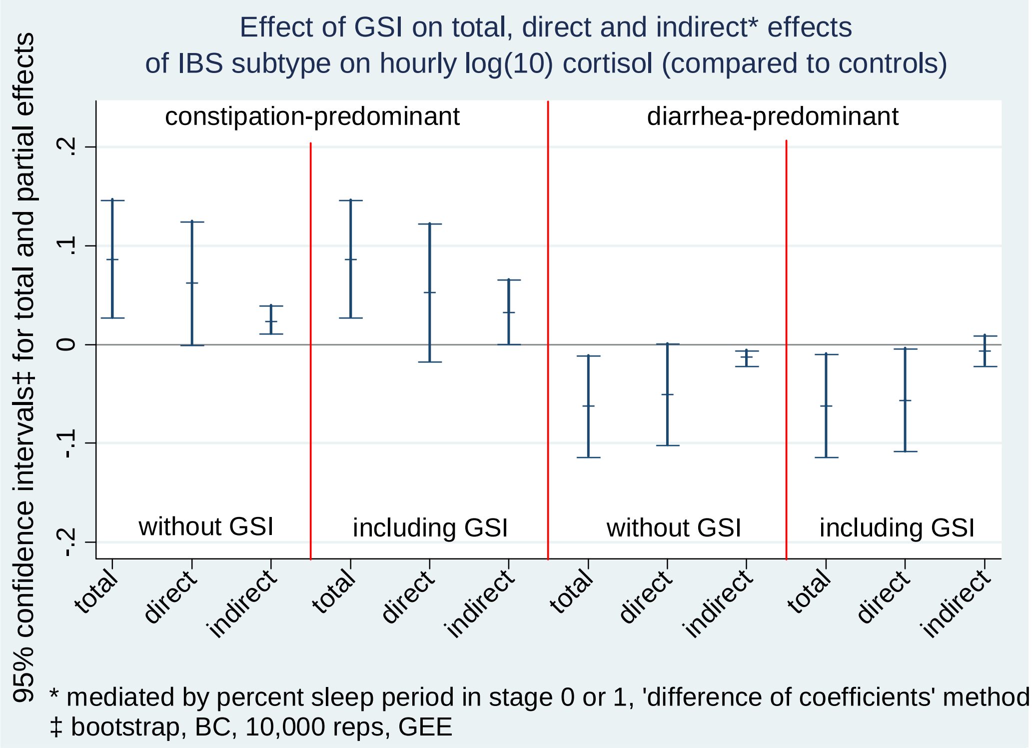


Figure 15 b). Confidence intervals for indirect effects of IBS subtype on nocturnal cortisol levels, with and without GSI in the mediation model.

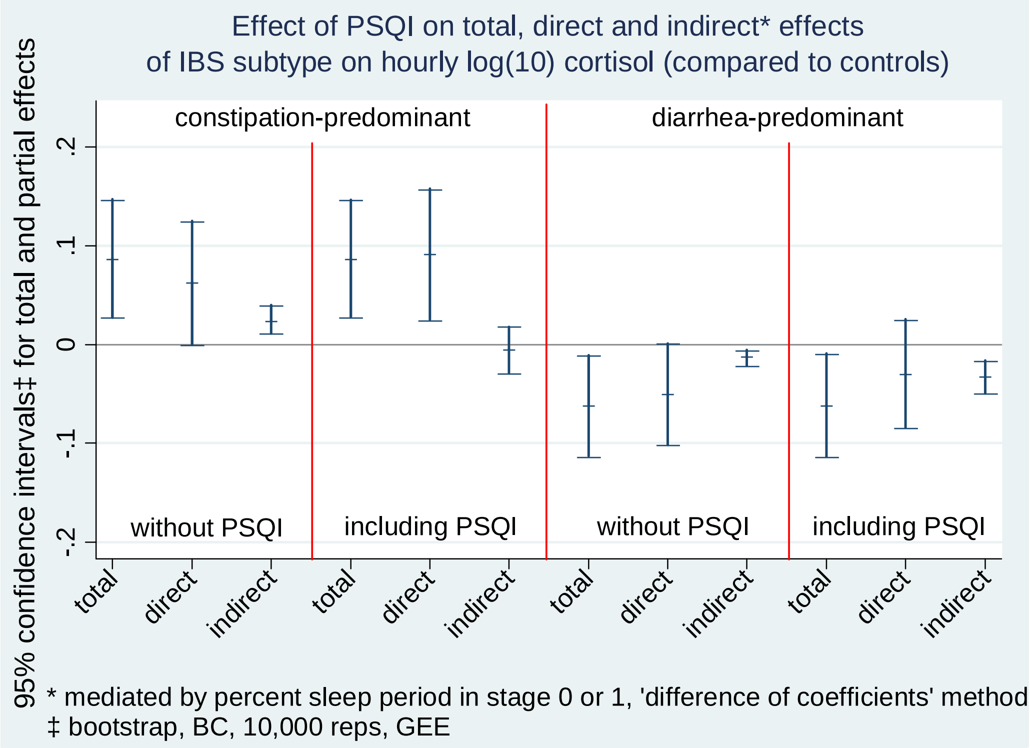


Figure 15 c). Confidence intervals for indirect effects of IBS subtype on nocturnal cortisol levels, with and without PSQI in the mediation model.

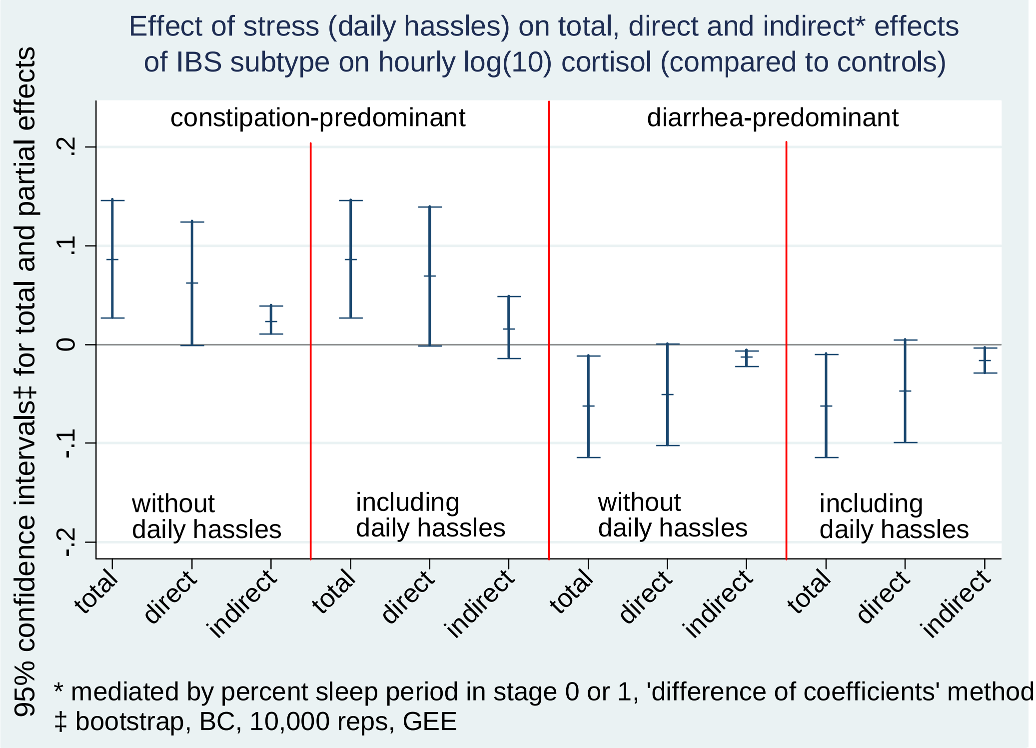


Figure 15 d). Confidence intervals for indirect effects of IBS subtype on nocturnal cortisol levels, with and without self-reported stress (daily hassles) in the mediation model.

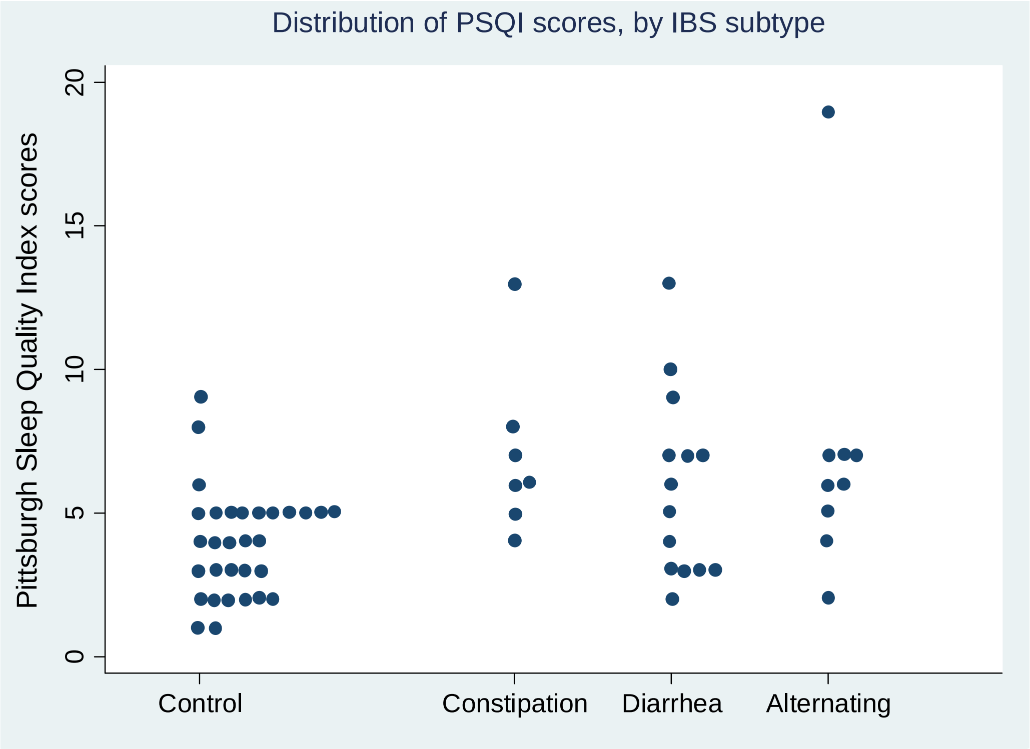


Figure 15 e). Distribution of PSQI scores across IBS subtypes.

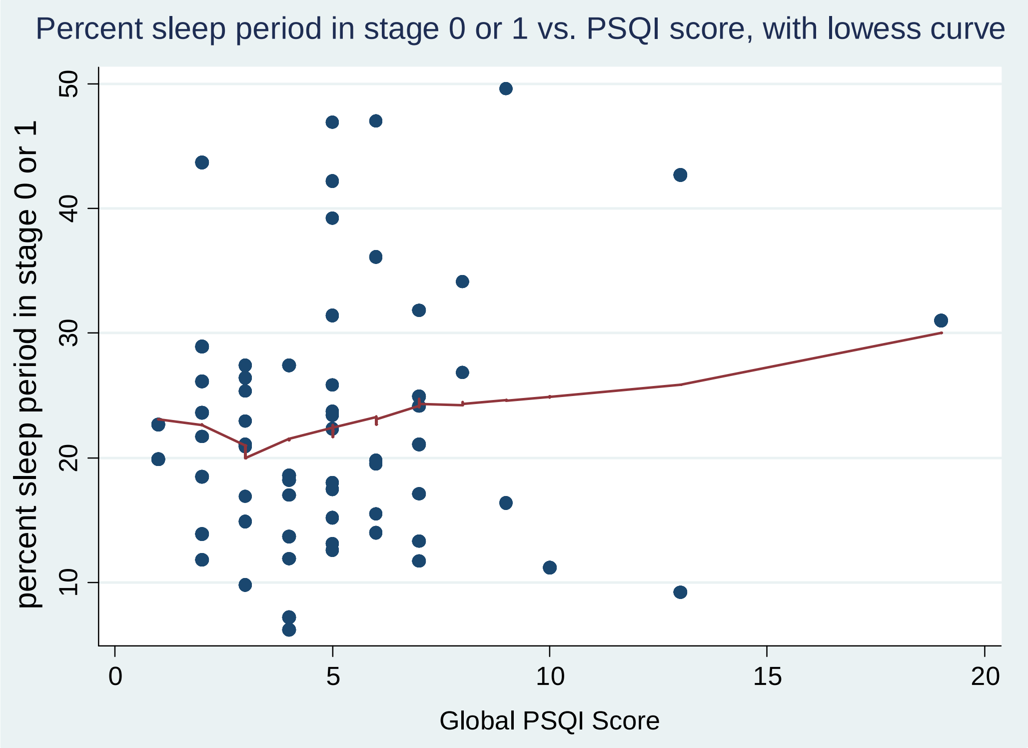
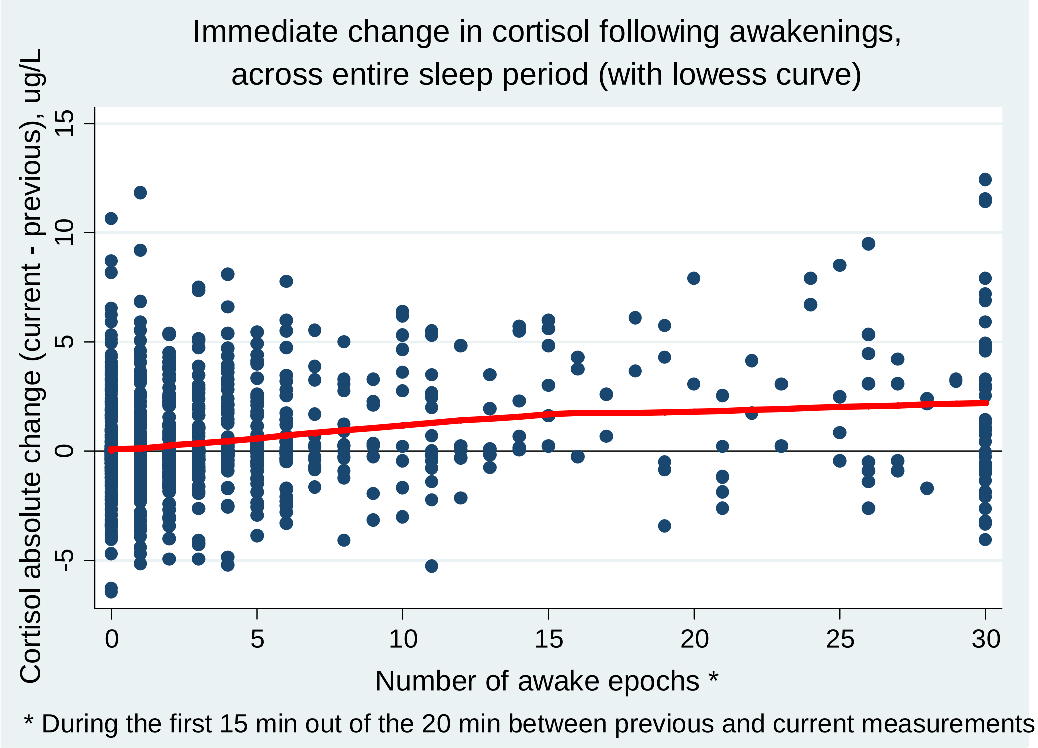


Figure 15 f). Percent sleep period in stage 0 or 1 vs. PSQI. Regression of percent sleep period in stage 0 or 1 vs. PSQI finds a small, positive but nonsignificant relationship ($p = 0.167$).

Relationship of individual awakenings to changes in cortisol levels

This analysis was restricted to time points after entering stage 2 sleep.

When the difference between each cortisol measurement and the prior measurement ($t_0 - t_{-1}$) is plotted against the number of awake 30-second epochs during the 15 minutes ending 5 minutes prior to the t_0 measurement (i.e., the 15 minutes immediately after the t_{-1} cortisol measurement), there appears to be a trend for immediate cortisol change to increase with increasing numbers of awake epochs (Figure 16). In broad terms, cortisol tends to rise after awakenings, and it tends to rise more after a greater number of awakenings in a short period of time. The analysis could not distinguish between contiguous and non-contiguous awake epochs, but as the number of awake epochs increases, the likelihood of longer contiguous awakenings increases. The trend appears to be most linear from 0 to 15 awake epochs, with a tendency to level off at higher numbers, mostly because of the sizeable number of points preceded by 30 awake epochs (i.e., awake for the entire 15 minutes, or more) where cortisol change was flat or declining. Results of a linear regression of ($t_0 – t_{-1}$) cortisol change on number of awake epochs after t_{-1}, including a variable for time in 30-second increments and per-subject clustering using robust standard errors, confirm the trend, with an estimated slope of .08 ug/L (95% CI: .05, .11) for each 30-second epoch increase in wakefulness. When the regression is restricted to ($t_0 – t_{-1}$) changes where a study nurse noted at or near t_0 that the subject awakened (Figure 17), the trend is even more pronounced, with a slope of .155 ug/L for each 30-second epoch increase in wakefulness (95% CI: .001, .310). One thing to note in Figure 17 is that about 1/3 of the nurse-noted awakenings had 0 prior awake epochs. Some of these were awakenings where the nurse immediately noted that the subject woke up (due to pain at the IV site, needing to use the bathroom, etc.). Some were cases where the nurse actually awakened the subject, e.g., while trying to reposition the IV line.



* During the first 15 min out of the 20 min between previous and current measurements

Figure 16. Increasing trend in immediate cortisol change ($t_0 - t_{-1}$) with increasing numbers of 30-second awake epochs between measurements. Slope calculated by linear regression is .079 ug/L for each 30-second epoch increase in wakefulness (95% CI: .047, .111).

# of awake epochs	0	1	2	3	4	5	6	7-10	11-15	16-20	21-25	26-29	30
cortisol change	0.1	0.0	0.3	0.4	0.8	0.6	0.8	1.0	1.4	2.6	1.9	1.9	2.0
mean (sd)	(1.9)	(2.2)	(2.0)	(2.3)	(2.5)	(2.1)	(2.6)	(2.5)	(2.6)	(3.1)	(3.4)	(3.2)	(4.3)

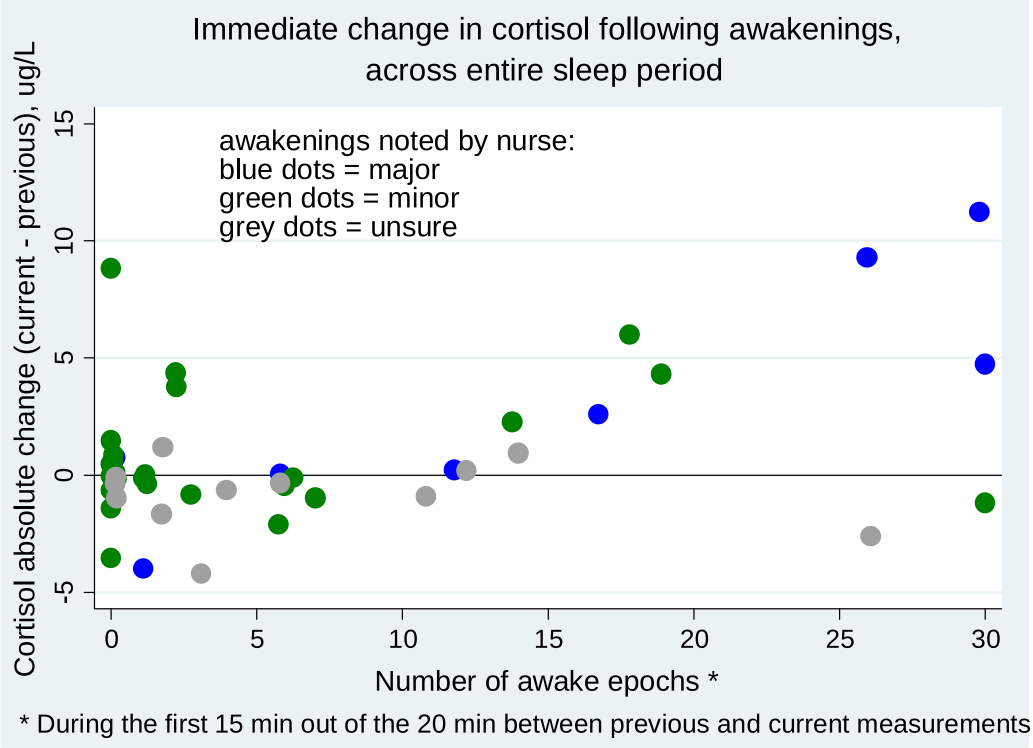


Figure 17. Increasing trend in immediate cortisol change ($t_0 - t_{-1}$) with increasing numbers of 30-second awake epochs between measurements, restricting awakenings to those noted by a study nurse at t_0. Slope calculated by linear regression is .155 ug/L for each 30-second epoch increase in wakefulness (95% CI: .001, .310).

When delayed change in cortisol ($t_{+1} - t_0$) is plotted against the number of awake 30-second epochs ending 5 minutes prior to the t_0 measurement, it appears that delayed cortisol change tends to decrease with increasing numbers of awake epochs (Figure 18). In broad terms, after the initial rise seen in Figures 16 and 17, cortisol tends to decrease, and a larger number of awake epochs is associated first with a higher increase and then with a larger decrease. Linear regression results confirm the trend and find that it is of similar magnitude to the initial increase, with a negative slope of -.055 ug/L (95% CI: .-.038, -.072) for each 30-second epoch increase in wakefulness. Interestingly, when the analysis is restricted to ($t_{+1} - t_0$) changes where a study nurse noted at or near t_0 that the subject awakened, the delayed decrease essentially disappears

(Figure 19), with a calculated slope of -.015 (95% CI: -.125, .095). This could be due to chance, or it could suggest that after more severe awakenings, cortisol tends to remain elevated rather than undergoing a compensatory drop. Individual trajectories of cortisol measurements taken prior to, just after, and following nurse-noted awakenings at t_0 are included in Appendix A.

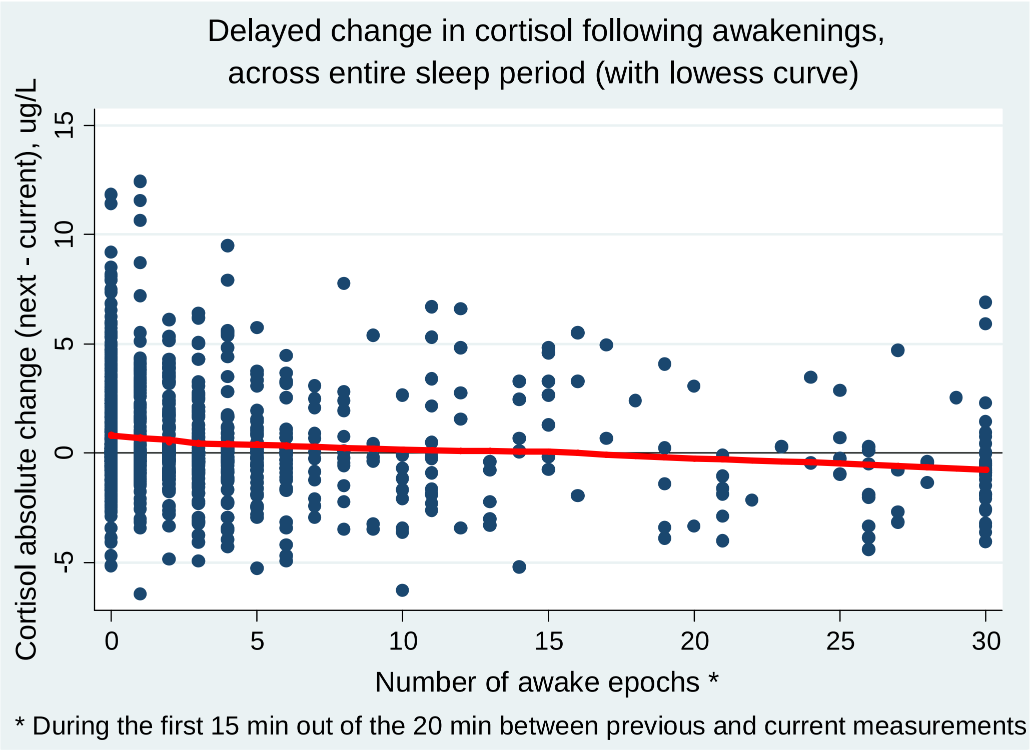


Figure 18. Decreasing trend in delayed cortisol change ($t_{+1} – t_0$) with increasing numbers of 30-second awake epochs between measurements. Slope calculated by linear regression is -.055 ug/L for each 30-second epoch increase in wakefulness (95% CI: -.038, -.072).

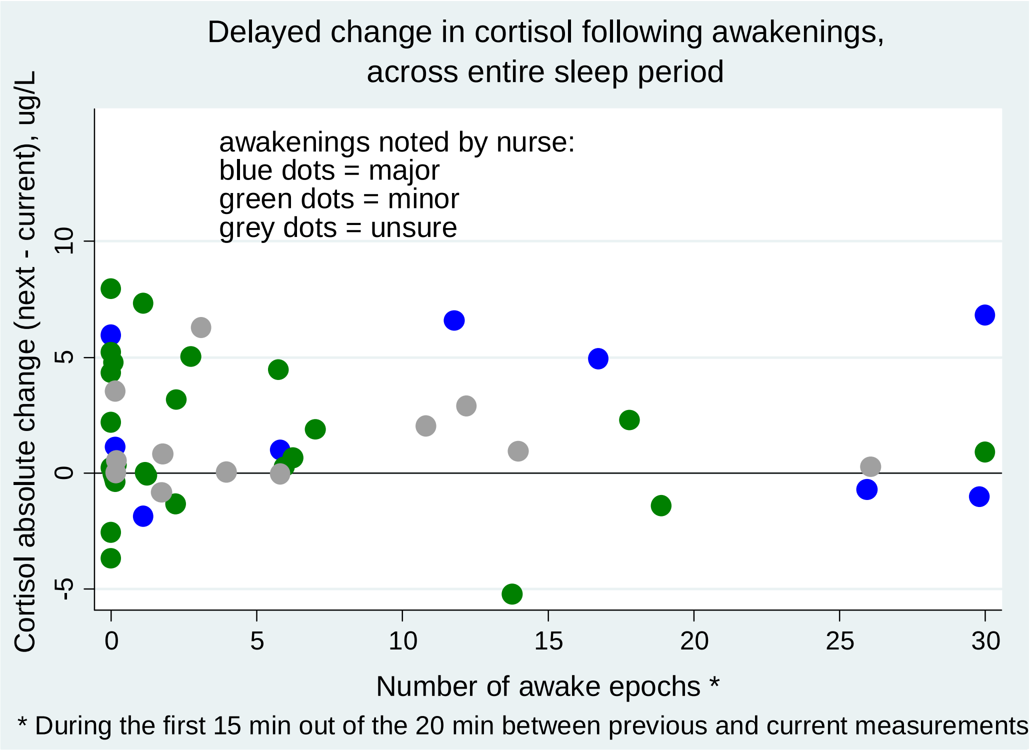


Figure 19. Trend in delayed cortisol change ($t_{+1} - t_0$) with increasing numbers of 30-second awake epochs between measurements, restricting awakenings to those noted by a study nurse at t_0. Slope calculated by linear regression is -.015 ug/L for each 30-second epoch increase in wakefulness (95% CI: -.125, .095).

The relationship between awakenings and cortisol change seems to vary depending on sleep period timing (Figures 20 and 21). When the previous analysis is repeated for early, middle and late sleep, it becomes apparent that the leveling off seen in the first plot of immediate change (Figure 17) is limited to the end of the sleep period (Figure 20). In this sample, mean cortisol change was highest from hour 4 to hour 5 after the onset of stage 2 sleep; during the last three hours, mean cortisol change was still positive but lower, as many subjects reached and then passed their cortisol peak (see Figure 7). This suggests that responsiveness to longer awakenings may be reduced in late sleep as cortisol levels flatten out or decline.

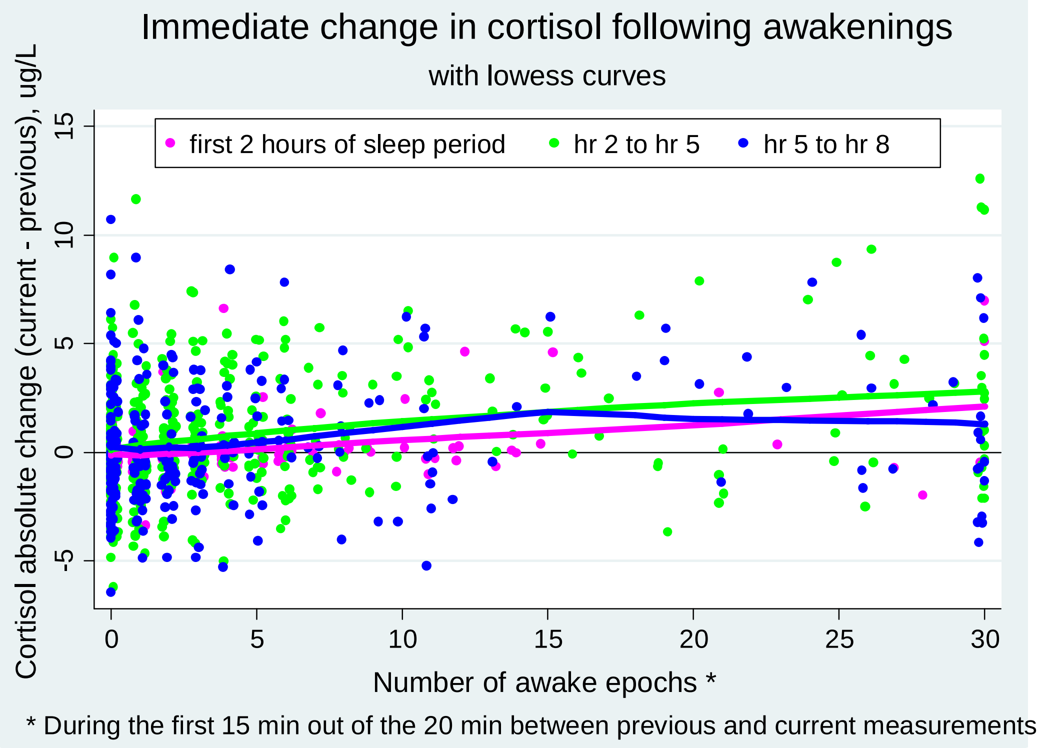


Figure 20. Increasing trend in immediate cortisol change $(t_0 - t_{-1})$ with increasing numbers of 30-second awake epochs between measurements, during early, middle and late sleep. The table below shows mean unconditional cortisol change during each hour after entering stage 2 sleep.

hours after entering S2 sleep	0-1	1-2	2-3	3-4	4-5	5-6	6-7	7-8
n	151	161	161	161	156	144	98	26
cortisol change, mean (sd)	-0.2 (0.8)	0.1 (1.2)	0.7 (2.1)	0.6 (2.6)	0.9 (3.0)	0.6 (3.1)	0.2 (2.7)	0.4 (2.3)

Differences in delayed cortisol response to awakenings based on sleep timing are even more pronounced (Figure 21). In early sleep, it appears that even after a 20+ minute delay, awakenings continue to cause cortisol to rise. During late sleep, delayed cortisol declines steeply with increasing awakenings, and during middle sleep the decline is more gradual.

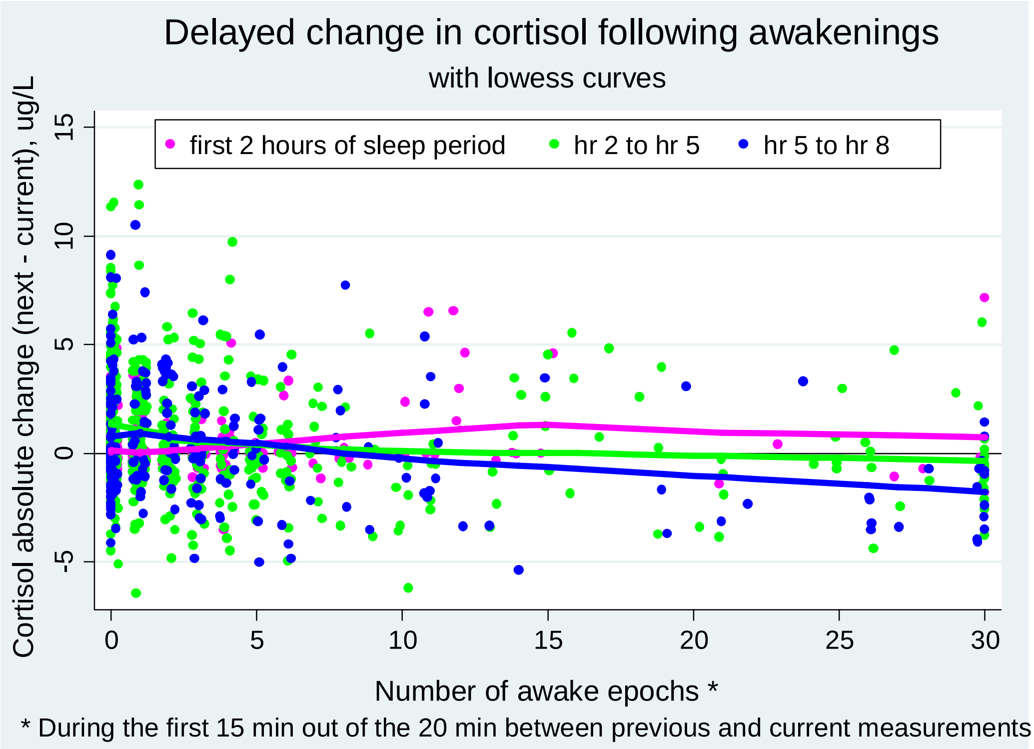


Figure 21. Decreasing trend in delayed cortisol change ($t_{+1} - t_0$) with increasing numbers of 30-second awake epochs between measurements, during early, middle and late sleep.

Figures 22-25 show the results of analyses exploring whether IBS subtype, age, BMI, and psychological distress modify the overall relationship between cortisol change and prior awakenings. Because these final analyses were considered exploratory and there were no a priori hypotheses about particular interactions, I did not perform any formal tests for interactions.

In Figure 22, there is some suggestion that the relationship may vary with IBS subtype; specifically, constipation-predominant and diarrhea-predeominant subjects may have a more pronounced rise in cortisol with 15 or fewer prior awake epochs, compared to alternating subjects or healthy controls, and the increasing cortisol response to high numbers of awake epochs may be reduced in constipation- and diarrhea-predominant subjects.

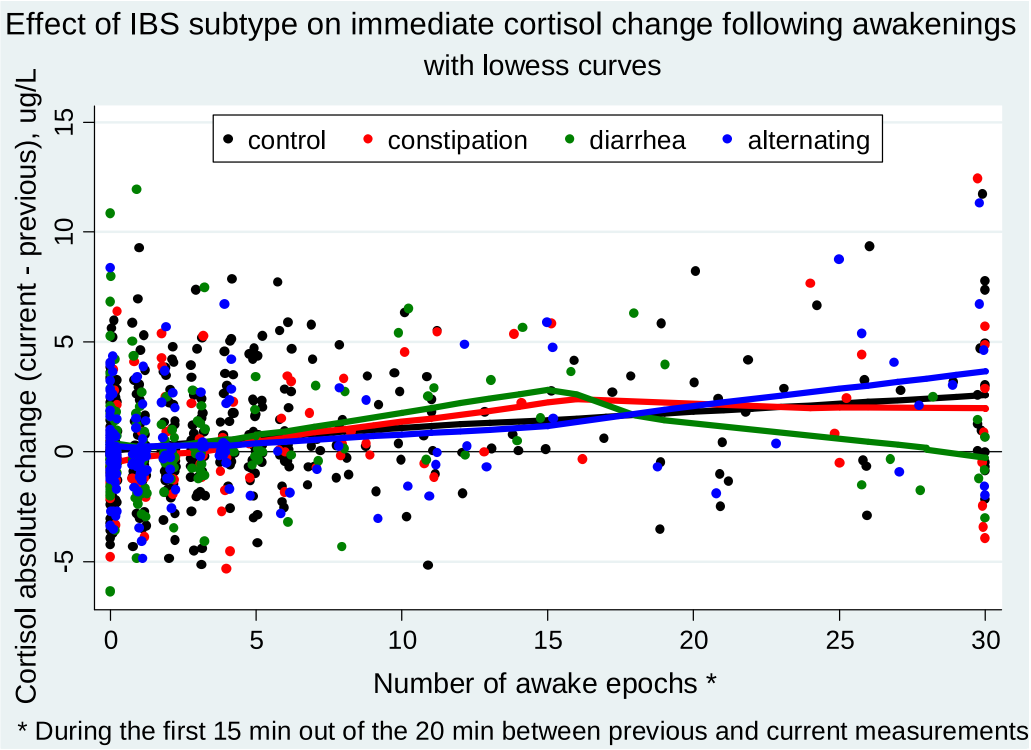


Figure 22. Effect of IBS subtype on the increasing trend in immediate cortisol change ($t_0 – t_{-1}$) with increasing numbers of 30-second awake epochs between measurements, during early, middle and late sleep.

In this sample, the age range was only 18-46, so there was a limited ability to investigate the effect of age on the awakenings-cortisol response, and the age results should be interpreted with considerable caution. That said, it appears (Figure 23) that there may be a tendency for older subjects (mid 30's and older) to have a cortisol response that is more sensitive to the high range of prior awake epoch counts, compared to younger subjects. By contrast, it appears that subjects reporting the highest levels of psychological distress may be less sensitive to the high range of prior awake epoch counts (Figure 25). BMI does not appear to be an effect modifier for the cortisol-awakening response in this sample (Figure 24).

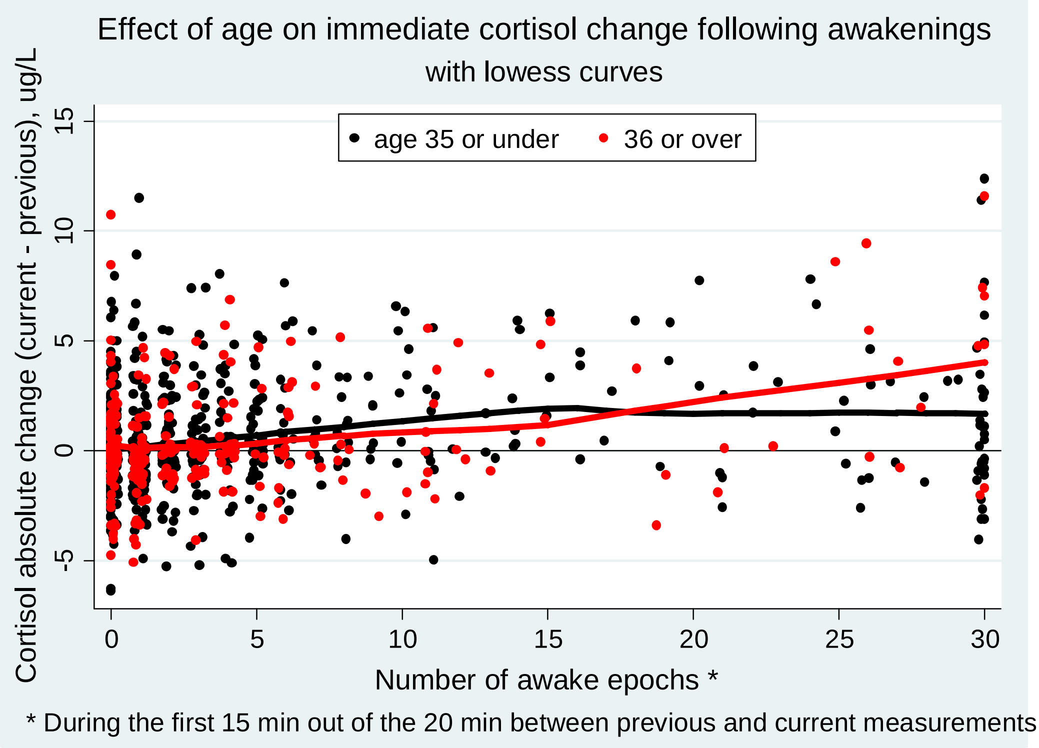


Figure 23. Effect of age on the increasing trend in immediate cortisol change ($t_0 - t_{-1}$) with increasing numbers of 30-second awake epochs between measurements, during early, middle and late sleep.

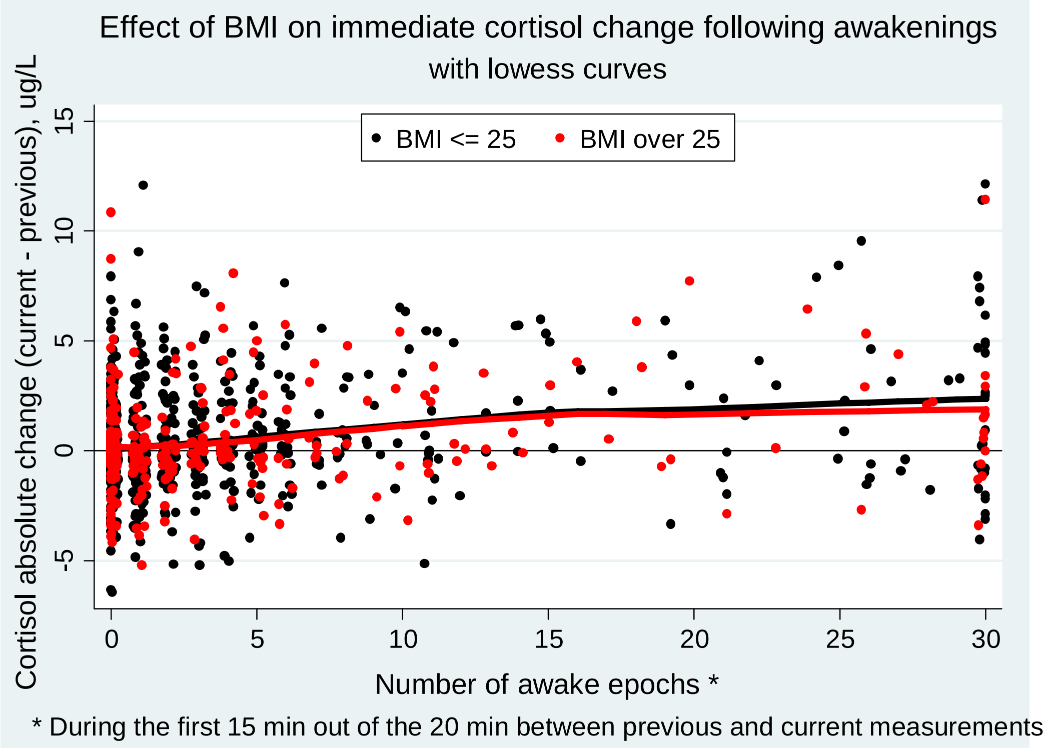


Figure 24. Effect of BMI on the increasing trend in immediate cortisol change ($t_0 - t_{-1}$) with increasing numbers of 30-second awake epochs between measurements, during early, middle and late sleep.

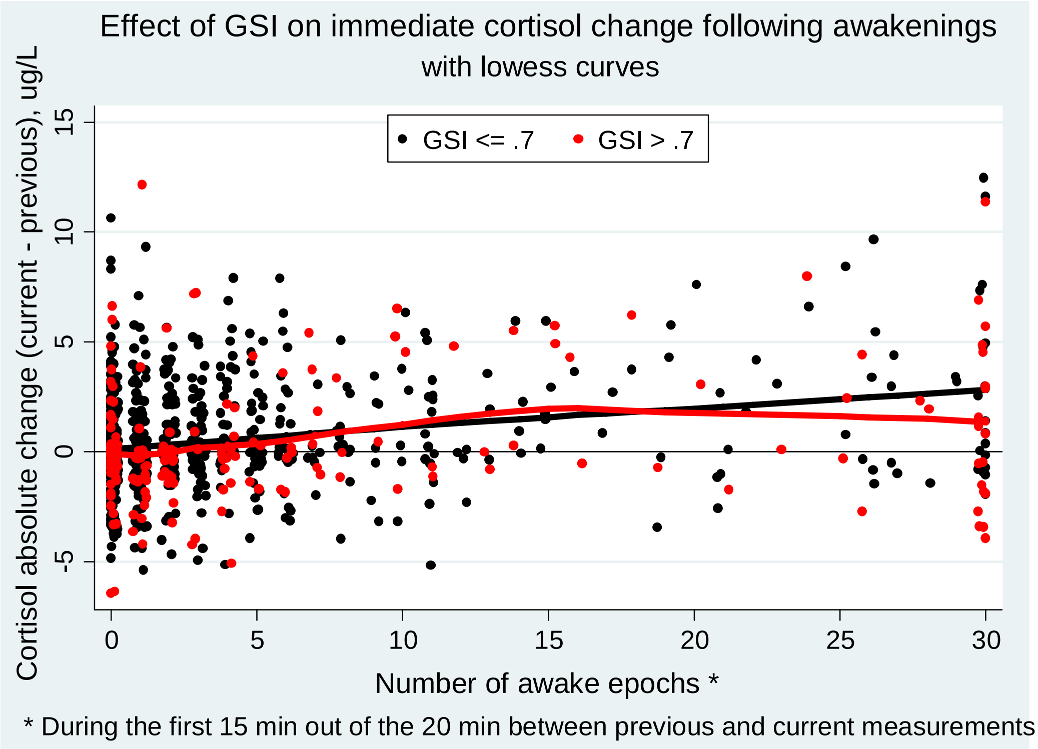


Figure 25. Effect of psychological distress on the increasing trend in immediate cortisol change ($t_0 - t_{-1}$) with increasing numbers of 30-second awake epochs between measurements, during early, middle and late sleep.

The last section of the analysis examined whether awakenings tended to be clustered before the steepest cortisol increases or after a local minimum, and whether fewer awakenings tended to be found before cortisol decreases. Figures 26 a) and b) present sample individual cortisol and sleep stage trajectories to show the pulsatile nature of serum cortisol excretion during sleep, including local minima and examples of nurse-noted awakenings. A complete panel of detailed plots is in Appendix C. It is possible to see awakenings (lowest level of red dots, stage 0) on individual plots, but drawing any clear and obvious pattern of awakenings relative to cortisol levels from inspecting individual plots is not possible. However, after categorizing prior awake epochs (0, 1-15, 16-29, and 30 epochs) and looking at positive and negative cortisol change and local minima, patterns do emerge. 17% of the 16-29 epoch awakenings and 22% of the 30-epoch awakenings are associated with the top 5% of cortisol changes, i.e., the steepest cortisol increases. Similarly, of the sleep periods preceding the steepest cortisol increases, only 15% had 0 awake epochs, compared to 38% with 0 awake epochs for the remaining cortisol changes. This certainly suggests that awakenings do tend to be clustered before the steepest cortisol increases. The pattern is reversed for negative cortisol changes; only 3% of the sleep periods preceding cortisol decreases had 16-29 and 30-epoch awakenings, while 39% of the preceding sleep periods had 0 awake epochs. This supports the hypothesis that fewer awakenings tend to be found before cortisol drops. There does not seem to be any tendency for awakenings to be clustered after local minima; the distribution of awake epochs after local minima does not differ from the distribution for other points.

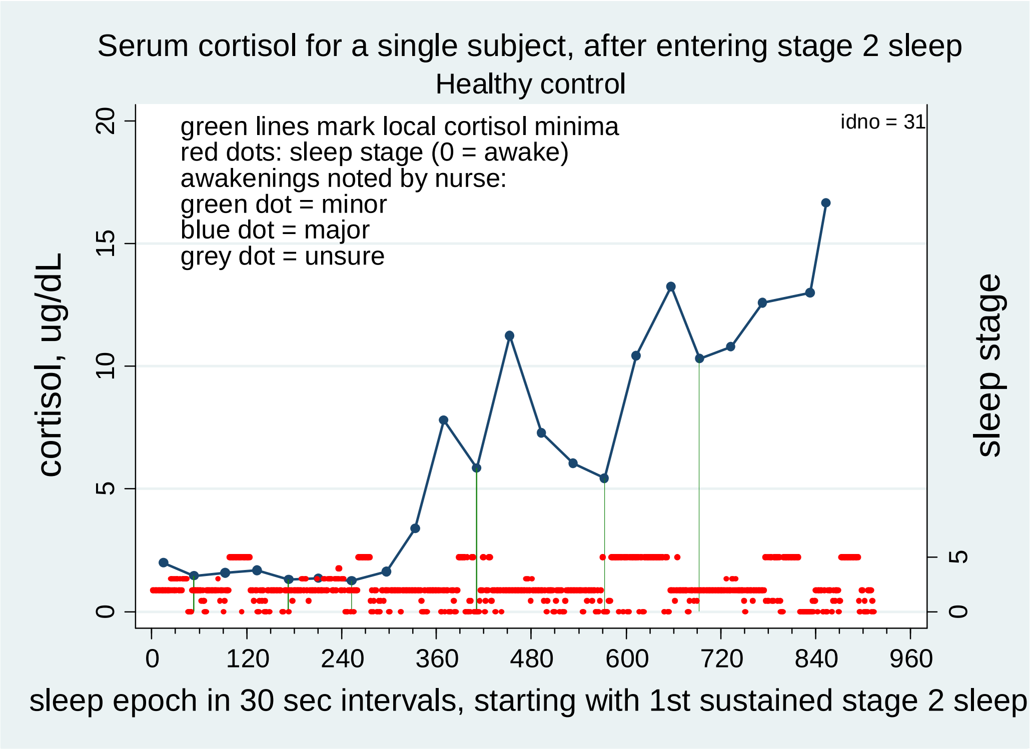


Figure 26 a). Individual time course for nocturnal plasma cortisol and sleep stages (in 30-second epochs) for a healthy control subject, showing pulsatility of cortisol excretion.

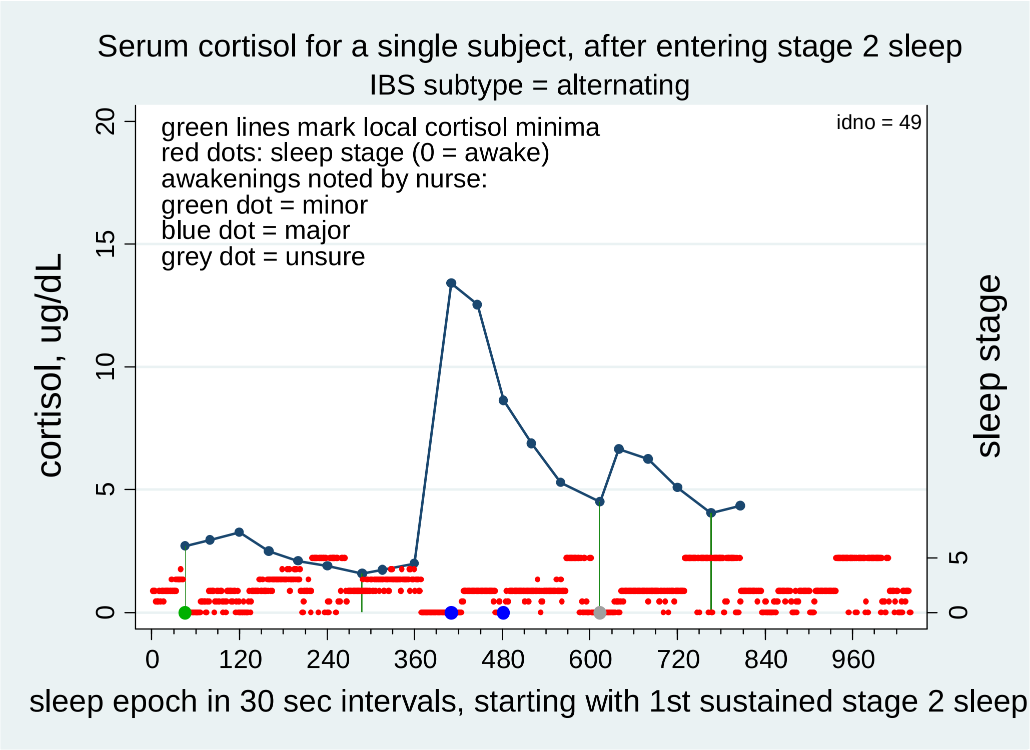


Figure 26 b). Individual time course for nocturnal plasma cortisol and sleep stages (in 30-second epochs) for an IBS subject, showing nurse-noted awakenings in relation to cortisol levels.

Discussion

Sleep disruption and nocturnal cortisol levels are related in this sample of young and middle-aged women. Compared to healthy controls, nocturnal cortisol levels were higher in constipation-predominant subjects with IBS and lower in diarrhea-predominant subjects with IBS, and a mediation analysis found small but significant indirect effects when sleep disruption was used as a mediator between IBS and $\log_{10}$(cortisol). The analysis cannot exclude the possibility that causality runs in the other direction, from sleep disruption to IBS subtype. For both healthy controls and subjects with IBS, individual awakenings were temporally linked to cortisol changes, with cortisol tending to increase following awakenings and then flatten out or decrease. This secretory pattern is consistent with the understanding that negative feedback following stress-related cortisol bursts is part of a homeostatic mechanism to keep 24-hour cortisol levels within a normal range.[1–3]

Given the complex and contradictory nature of the literature on relationships between sleep and cortisol in stress-related disorders, it is noteworthy that in this sample, sleep disruption clearly increased the association of IBS with nocturnal cortisol, but that association was different for different IBS subtypes. For constipation-predominant women, sleep disruption was associated with higher cortisol levels relative to controls, while for diarrhea-predominant women, sleep disruption was associated with lower cortisol relative to controls. When IBS subjects were compared as a heterogeneous single group to controls, both the estimated total effect and the estimated indirect effect mediated by sleep approached zero.

I emphasize the finding that sleep-mediated indirect effects exist and that confidence intervals excluded zero for two out of three IBS subtypes, rather than any specific effect estimates, because of the small sample size, especially in the IBS subtypes. There were 29 healthy controls and 29 women with IBS, including 7 constipation-predominant, 13 diarrhea-predominant, and 9 alternating subjects. IBS is a syndrome quite variable in symptom severity, comorbid psychiatric disorders, and likely in etiology, so results from a small single sample should be interpreted with caution.[4] In addition, ‘sleep disruption’ is a complex phenomenon in itself, with multiple components including number, duration, and timing of arousals, among others. The two summary measures examined here – percent sleep period in stage 0 or 1, and a per-hour fragmentation index – gave slightly different results in the mediation analysis, suggesting that nocturnal HPA activation in subjects with IBS may not respond uniformly to all aspects of sleep disruption. This is supported by the finding in this analysis that the relationship between awakenings and cortisol change seems to vary depending on sleep period timing. In early sleep, the stimulatory effect of awakenings lasts longer than during middle or late sleep. During late sleep, when cortisol is rising toward its peak or already declining, there appears to be a threshold beyond which longer awakenings (up to 15 minutes) are no more stimulatory than shorter awakenings. Throughout the night, awakenings tended to precede cortisol spikes rather than the other way around, with a short latency period of 20 minutes or less.

Two major limitations of this study were the small sample size and the fact that study participants with IBS were self-selected from a community-based population, both of which may limit the generalizability of the results. Missing data were probably not influential in this analysis; only two subjects (one control, one IBS-D) out of 58 had fewer than 6 hours of sleep and cortisol data, and there was little difference between the GEE and random-effects mixed longitudinal model results. One limitation of the data was that the actual time of awakenings noted by the study nurse was often uncertain, so the alignment between the nurse-noted awakening and the prior awake epochs may have been incorrect for a number of those events. Finally, I did not measure contiguous awakenings, so I could not analyze the actual relationship of length of awakenings to cortisol levels.

Conclusions – Mediation Analysis

These data are consistent with sleep disruption, as measured by the percent sleep period in stage 0 or 1, mediating some amount of an IBS effect on nocturnal cortisol in this sample of young and middle-aged women with IBS. This indirect effect appears to be present for both constipation-predominant and diarrhea-predominant IBS subtypes, but not for the alternating subtype. The indirect effects were consistent in direction with the total effect, suggesting that sleep disruption increases the net effect of IBS on nocturnal cortisol.

Conclusions – Temporal Relationship of Awakenings and Cortisol Changes

In this sample of young and middle-aged women with IBS, awakenings tend to be followed first by a rise in cortisol and then by a subsequent cortisol decrease. The magnitude of both rise and fall tend to increase with the number of 30-second awake epochs in a 15-minute period and vary with sleep period timing. In early sleep, the stimulatory effect of awakenings lasts longer than during middle or late sleep. During late sleep, when cortisol is rising toward its peak or already declining, there appears to be a threshold beyond which greater numbers of awake epochs exert no additional stimulatory effect. Awakenings tend to be distributed with more or longer awakenings before the steepest cortisol increases, and no or fewer awakenings before cortisol decreases.

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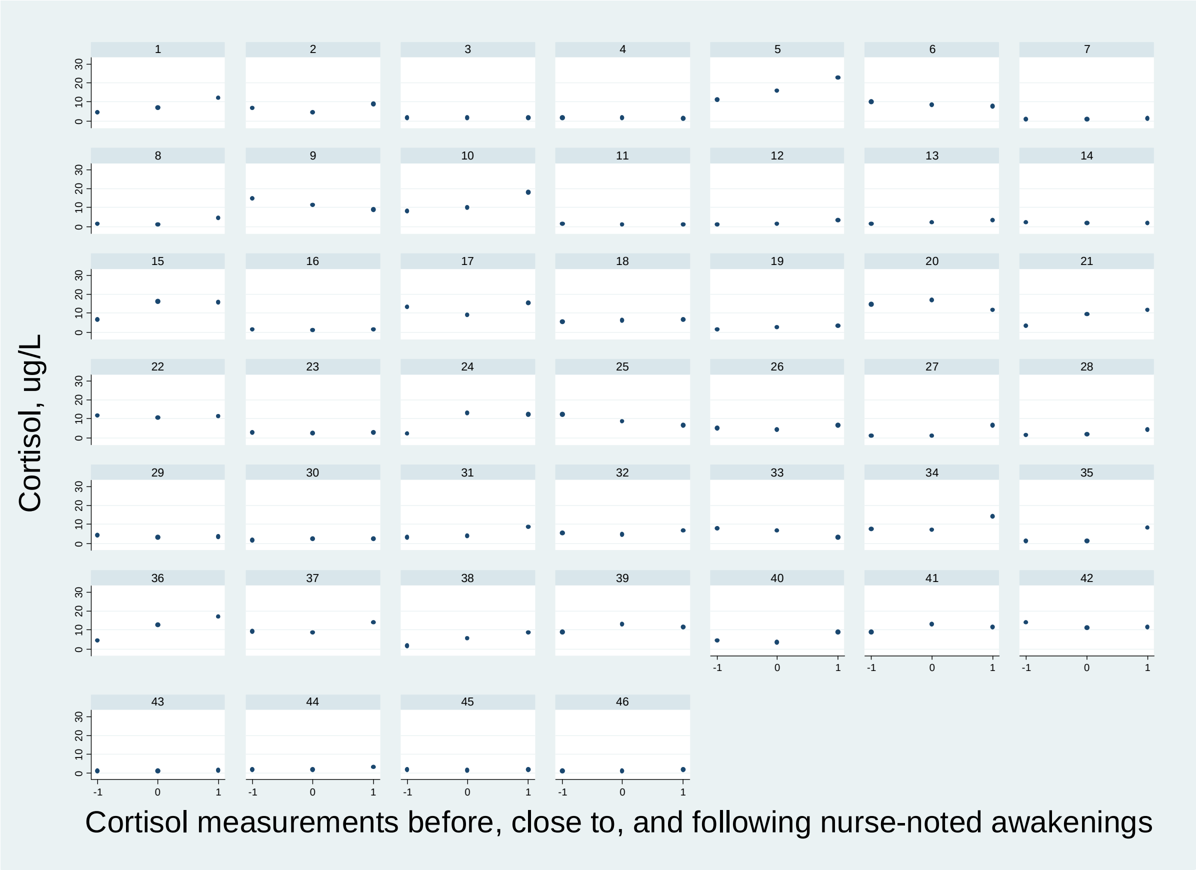
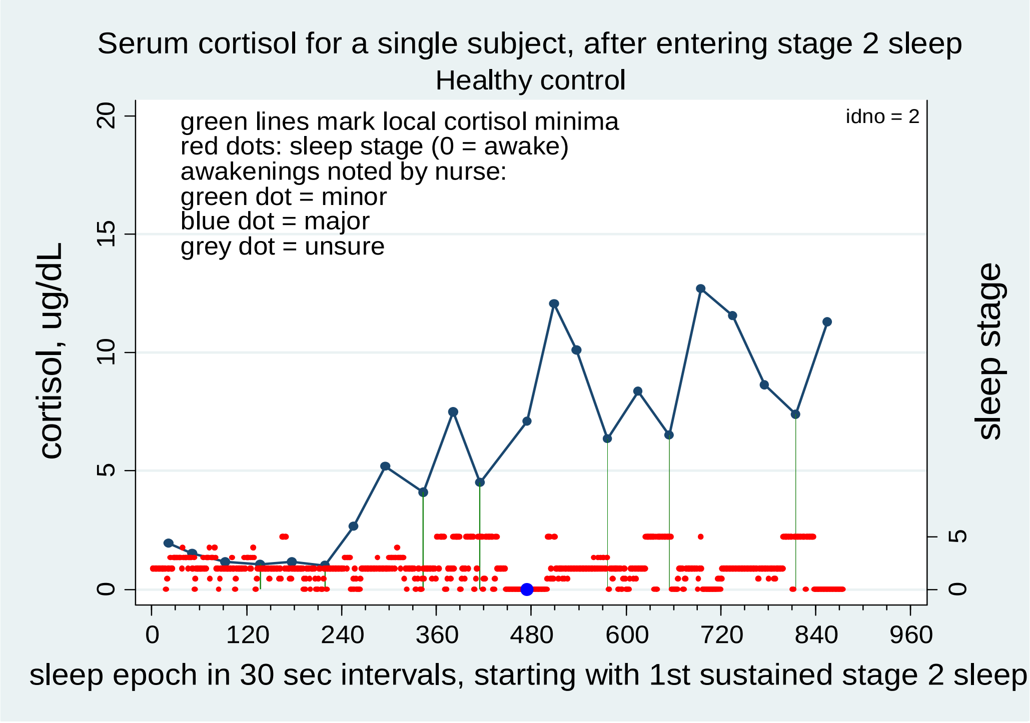
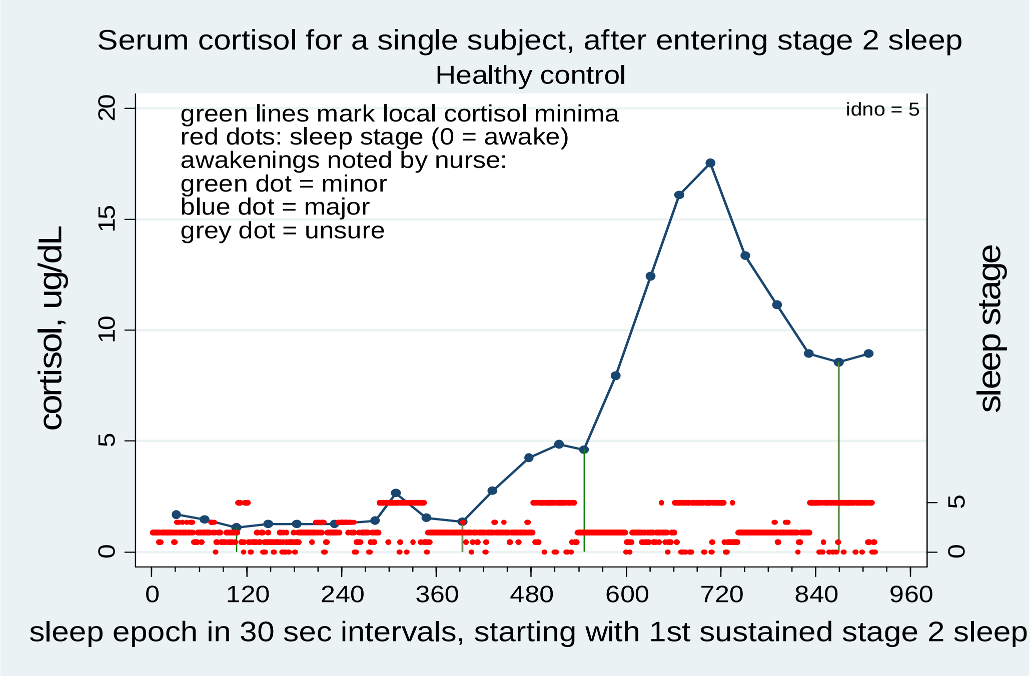
Appendix A: Cortisol measurements bracketing nurse-noted awakenings

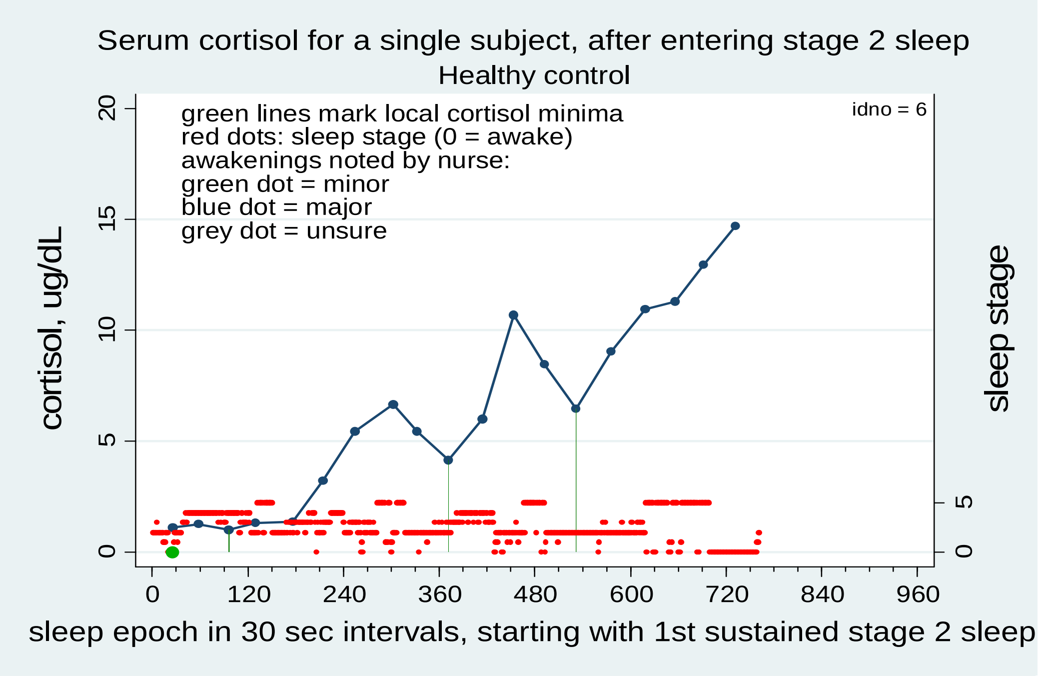


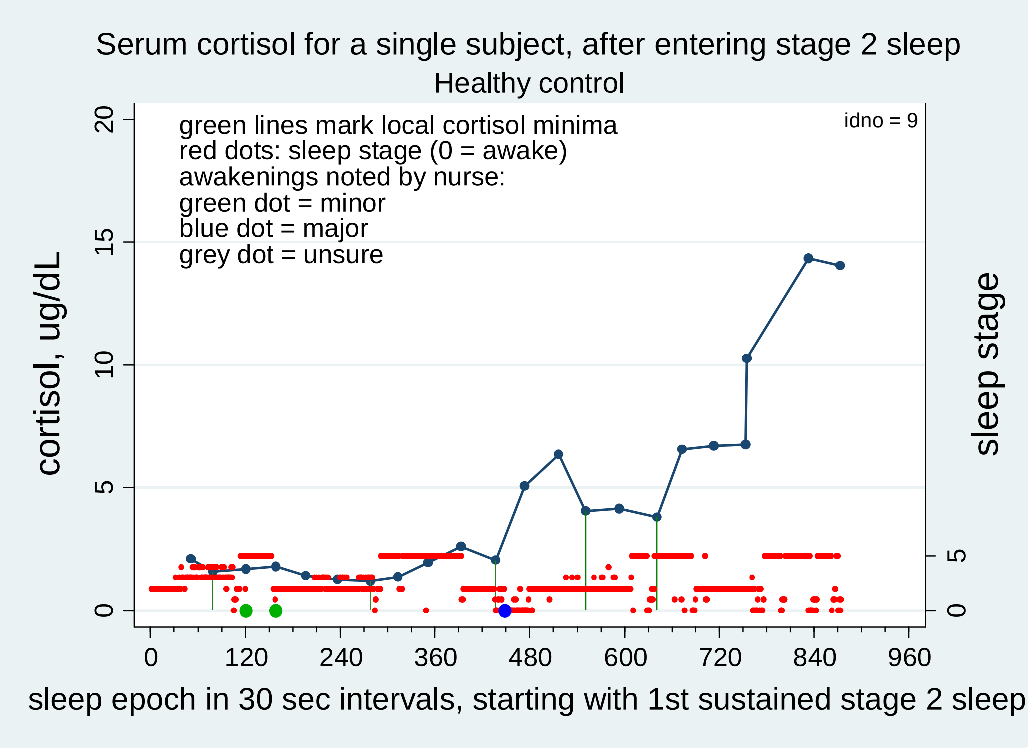
Appendix B: Detailed individual sleep and cortisol trajectories

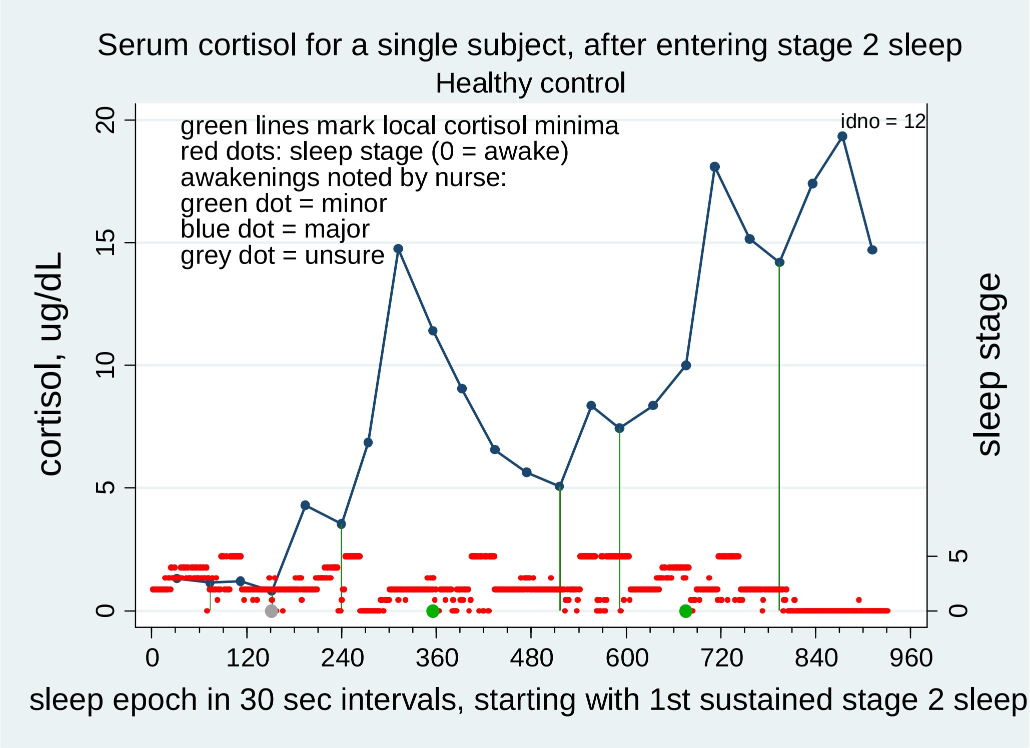
Healthy Controls

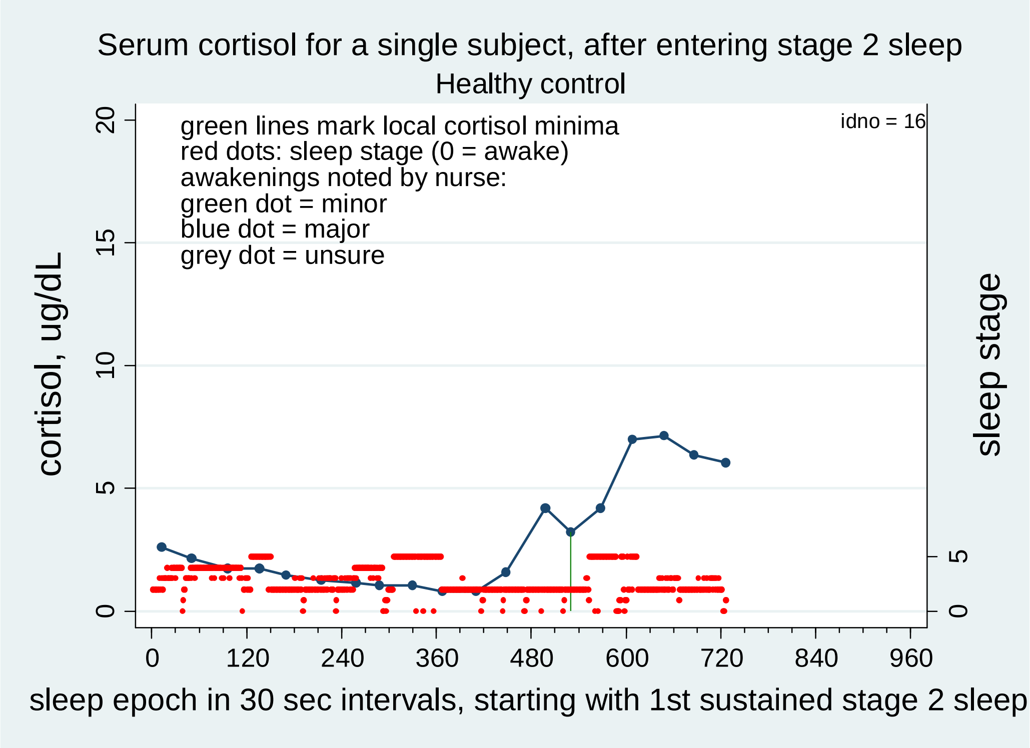


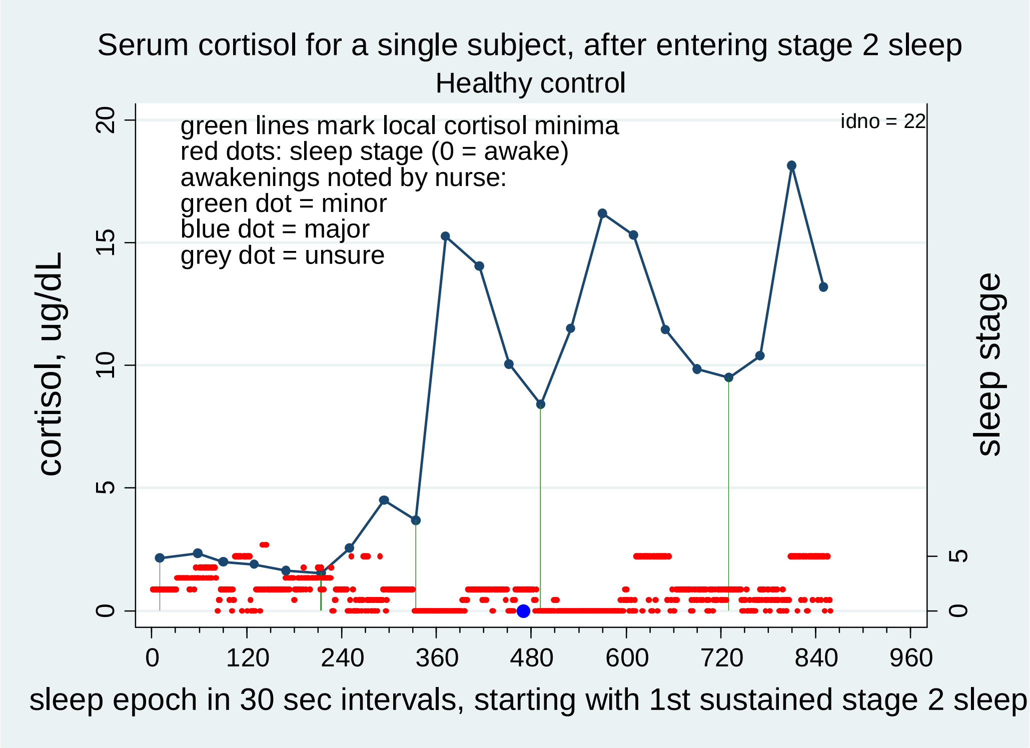


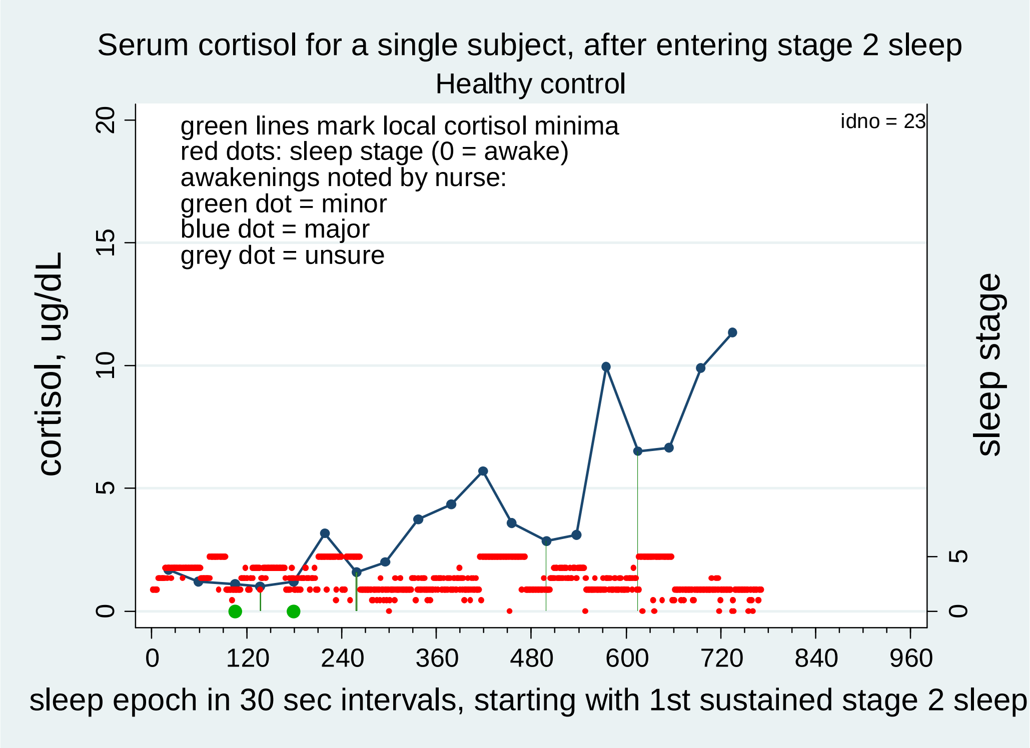

Serum cortisol for a single subject, after entering stage 2 sleep
Healthy control
green lines mark local cortisol minima
red dots: sleep stage (0 = awake)
awakenings noted by nurse:
green dot = minor
blue dot = major
grey dot = unsure
idno = 6
cortisol, ug/dL
0
5
10
15
20
sleep stage
0
5
0
120
240
360
480
600
720
840
960
sleep epoch in 30 sec intervals, starting with 1st sustained stage 2 sleep

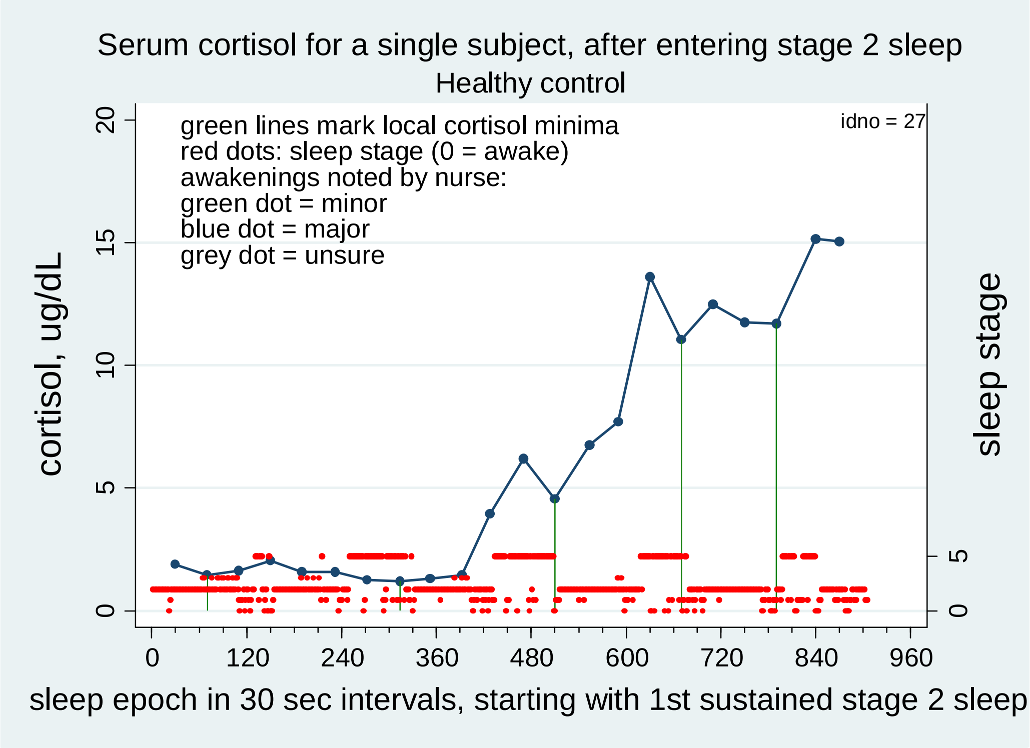

Serum cortisol for a single subject, after entering stage 2 sleep
Healthy control
green lines mark local cortisol minima
red dots: sleep stage (0 = awake)
awakenings noted by nurse:
green dot = minor
blue dot = major
grey dot = unsure
idno = 9
cortisol, ug/dL
0
5
10
15
20
sleep stage
0
5
0
120
240
360
480
600
720
840
960
sleep epoch in 30 sec intervals, starting with 1st sustained stage 2 sleep

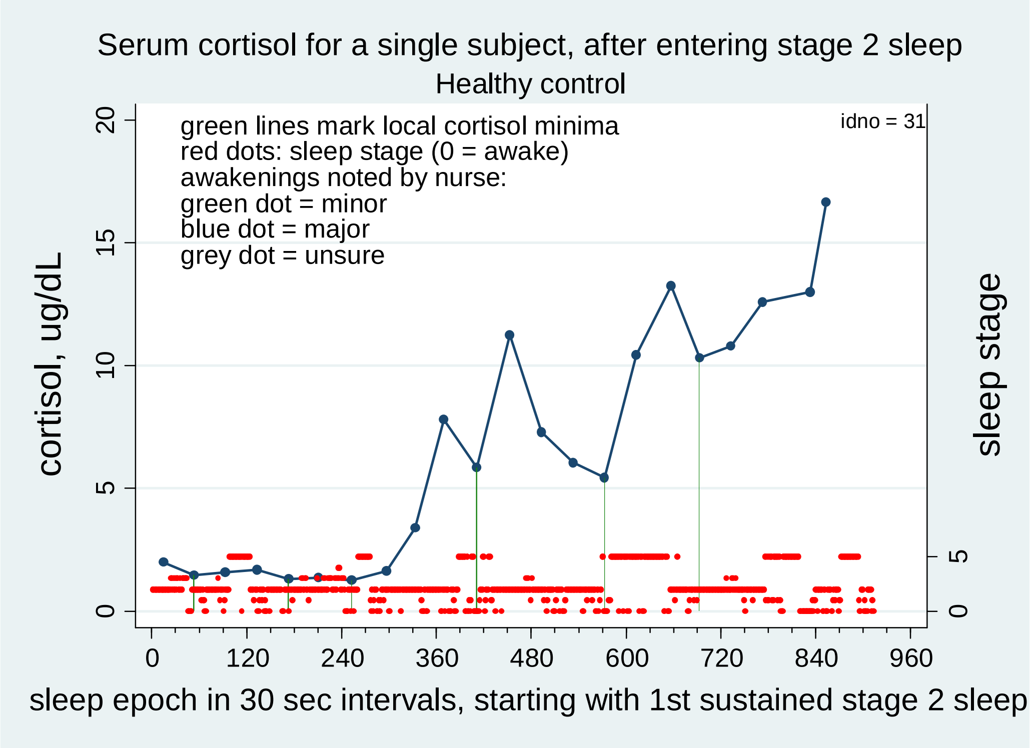

Serum cortisol for a single subject, after entering stage 2 sleep
Healthy control
green lines mark local cortisol minima
red dots: sleep stage (0 = awake)
awakenings noted by nurse:
green dot = minor
blue dot = major
grey dot = unsure
idno = 12
cortisol, ug/dL
sleep stage
0
5
10
15
20
0
5
0
120
240
360
480
600
720
840
960
sleep epoch in 30 sec intervals, starting with 1st sustained stage 2 sleep

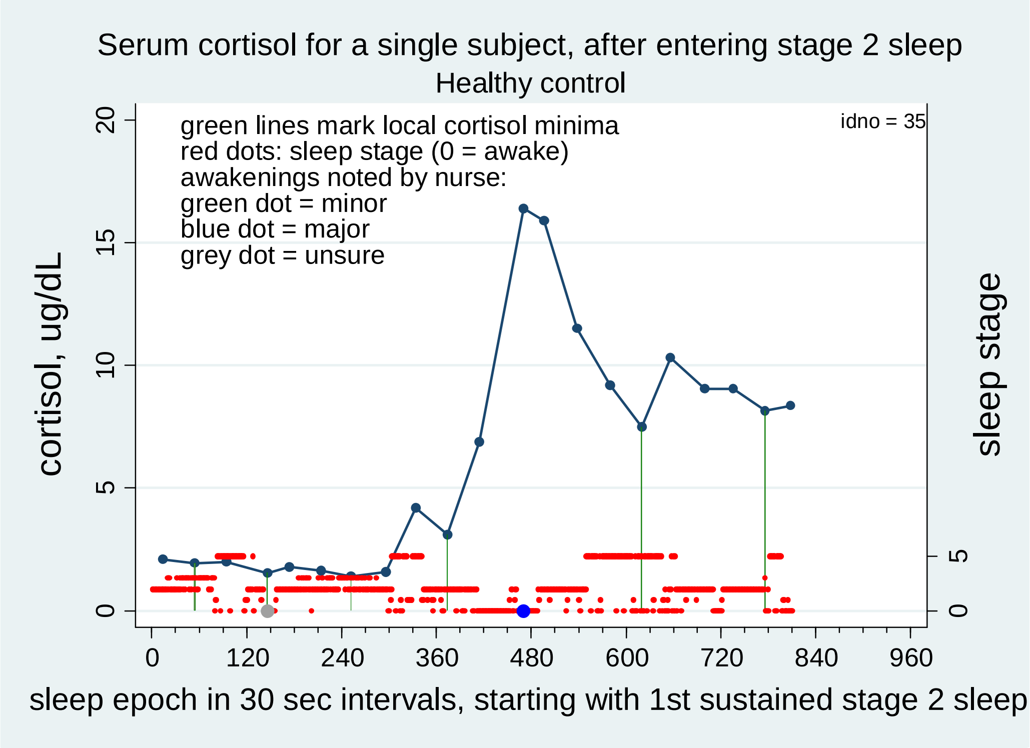

Serum cortisol for a single subject, after entering stage 2 sleep
Healthy control
green lines mark local cortisol minima
red dots: sleep stage (0 = awake)
awakenings noted by nurse:
green dot = minor
blue dot = major
grey dot = unsure
idno = 16
cortisol, ug/dL
sleep stage
0
5
10
15
20
0
5
0
120
240
360
480
600
720
840
960
sleep epoch in 30 sec intervals, starting with 1st sustained stage 2 sleep

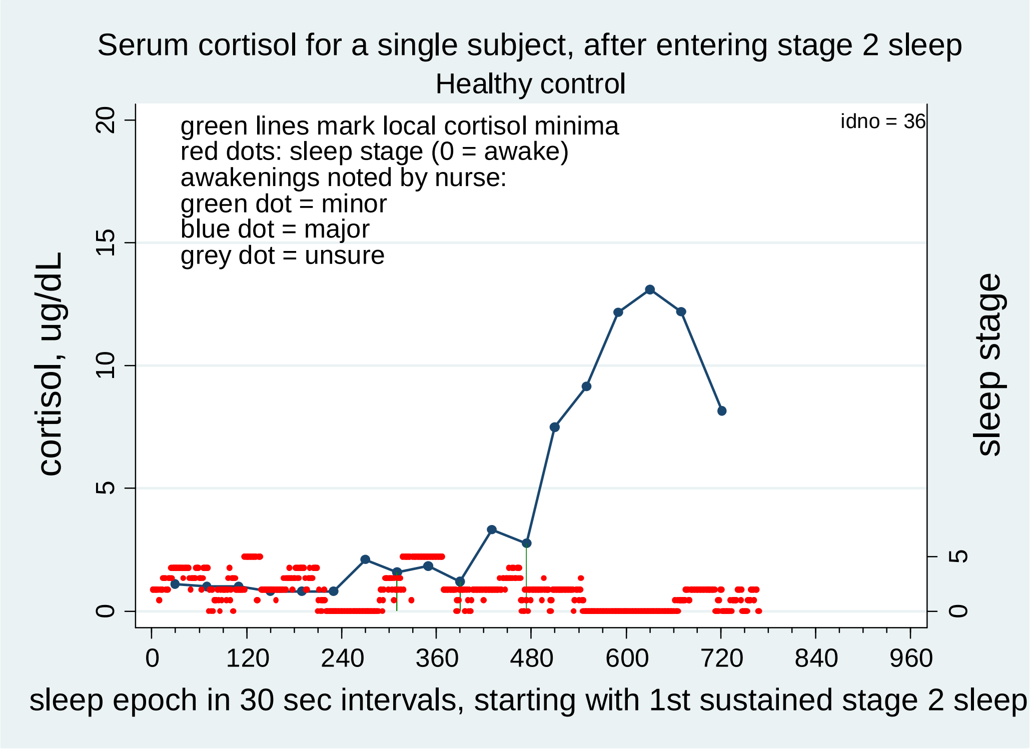

Serum cortisol for a single subject, after entering stage 2 sleep
Healthy control
green lines mark local cortisol minima
red dots: sleep stage (0 = awake)
awakenings noted by nurse:
green dot = minor
blue dot = major
grey dot = unsure
idno = 22
cortisol, ug/dL
0
5
10
15
20
sleep stage
0
5
0
120
240
360
480
600
720
840
960
sleep epoch in 30 sec intervals, starting with 1st sustained stage 2 sleep

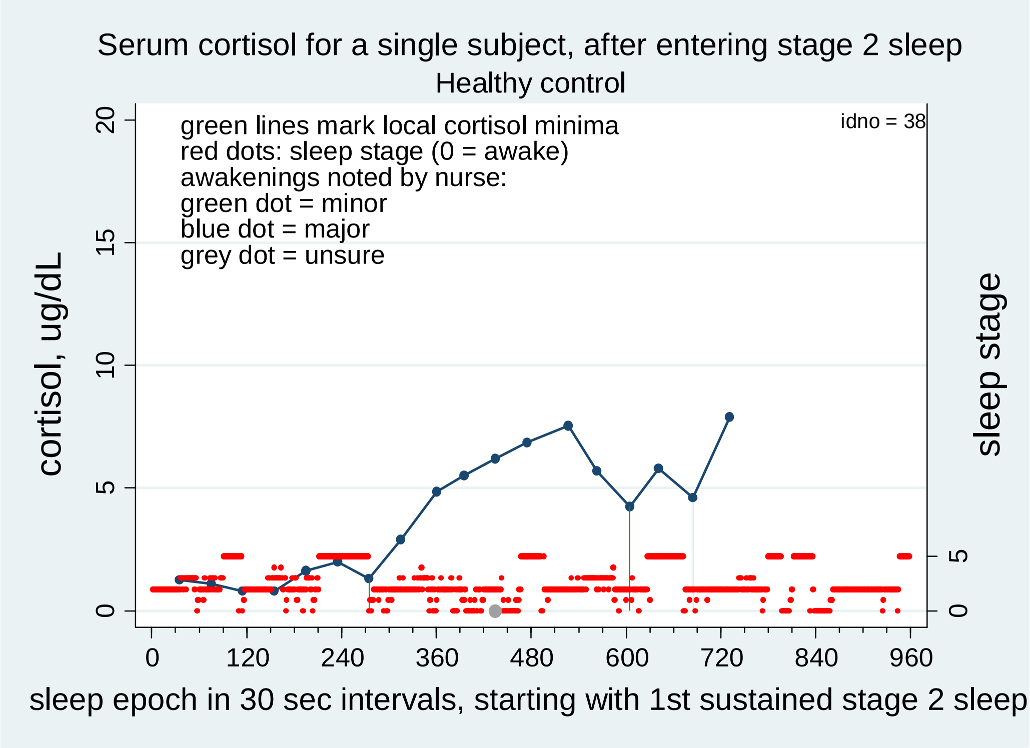

Serum cortisol for a single subject, after entering stage 2 sleep
Healthy control
green lines mark local cortisol minima
red dots: sleep stage (0 = awake)
awakenings noted by nurse:
green dot = minor
blue dot = major
grey dot = unsure
idno = 23
cortisol, ug/dL
0
5
10
15
20
sleep stage
0
5
0
120
240
360
480
600
720
840
960
sleep epoch in 30 sec intervals, starting with 1st sustained stage 2 sleep

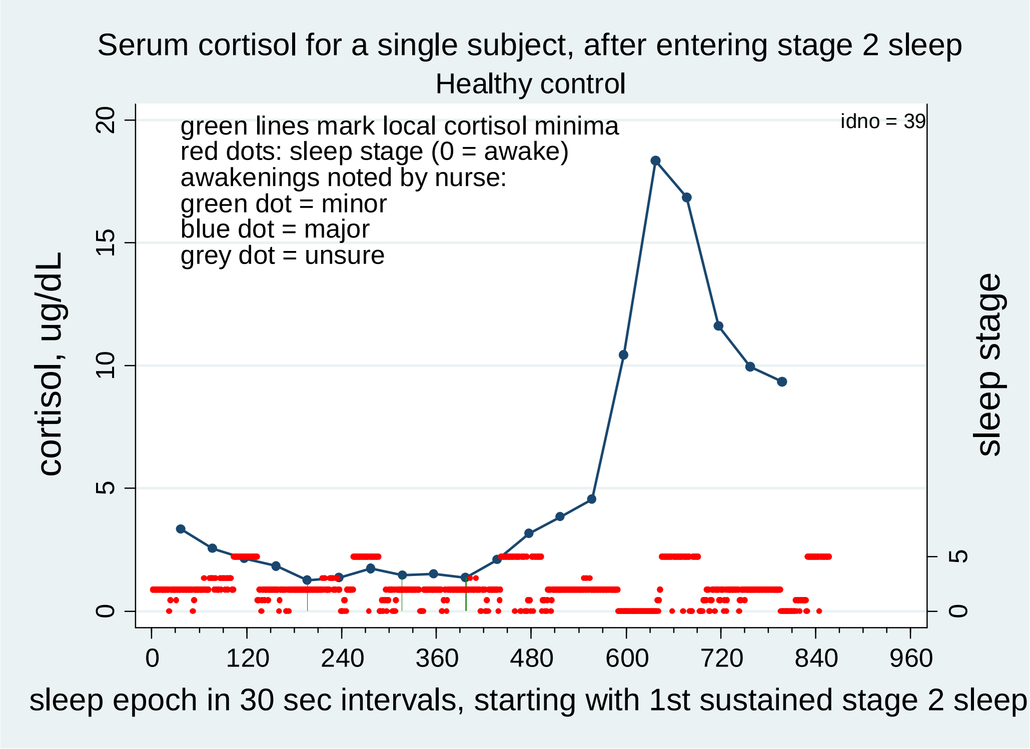

Serum cortisol for a single subject, after entering stage 2 sleep
Healthy control
green lines mark local cortisol minima
red dots: sleep stage (0 = awake)
awakenings noted by nurse:
green dot = minor
blue dot = major
grey dot = unsure
idno = 27
cortisol, ug/dL
0
5
10
15
20
sleep stage
0
5
0
120
240
360
480
600
720
840
960
sleep epoch in 30 sec intervals, starting with 1st sustained stage 2 sleep

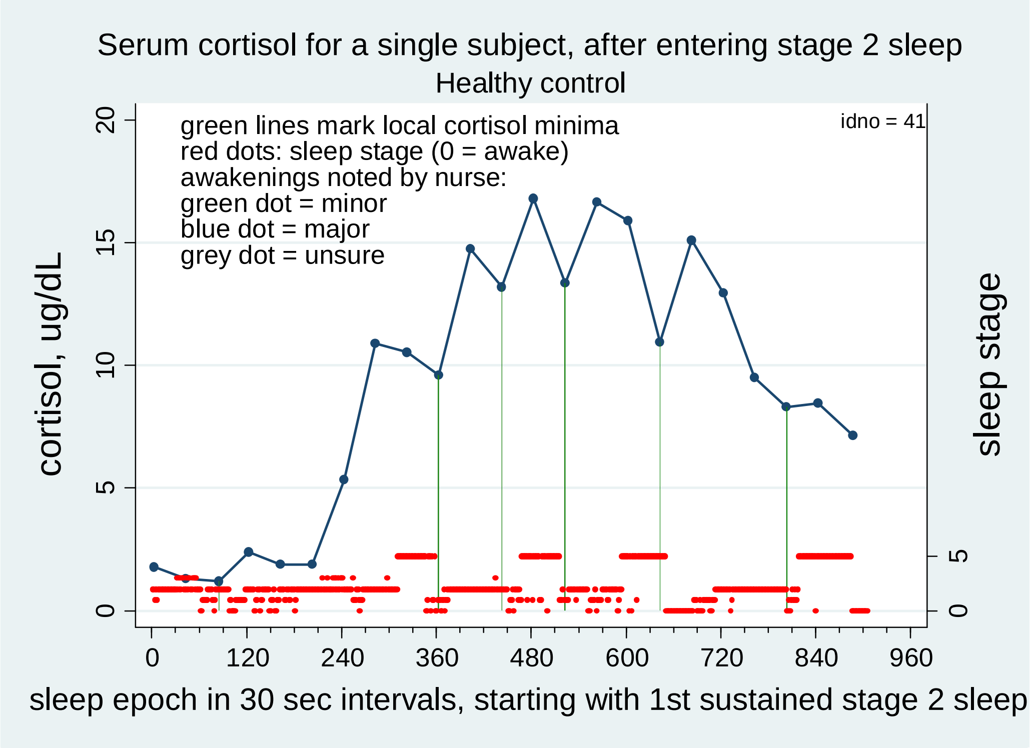

Serum cortisol for a single subject, after entering stage 2 sleep
Healthy control
green lines mark local cortisol minima
red dots: sleep stage (0 = awake)
awakenings noted by nurse:
green dot = minor
blue dot = major
grey dot = unsure
idno = 31
cortisol, ug/dL
0
5
10
15
20
sleep stage
0
5
0
120
240
360
480
600
720
840
960
sleep epoch in 30 sec intervals, starting with 1st sustained stage 2 sleep

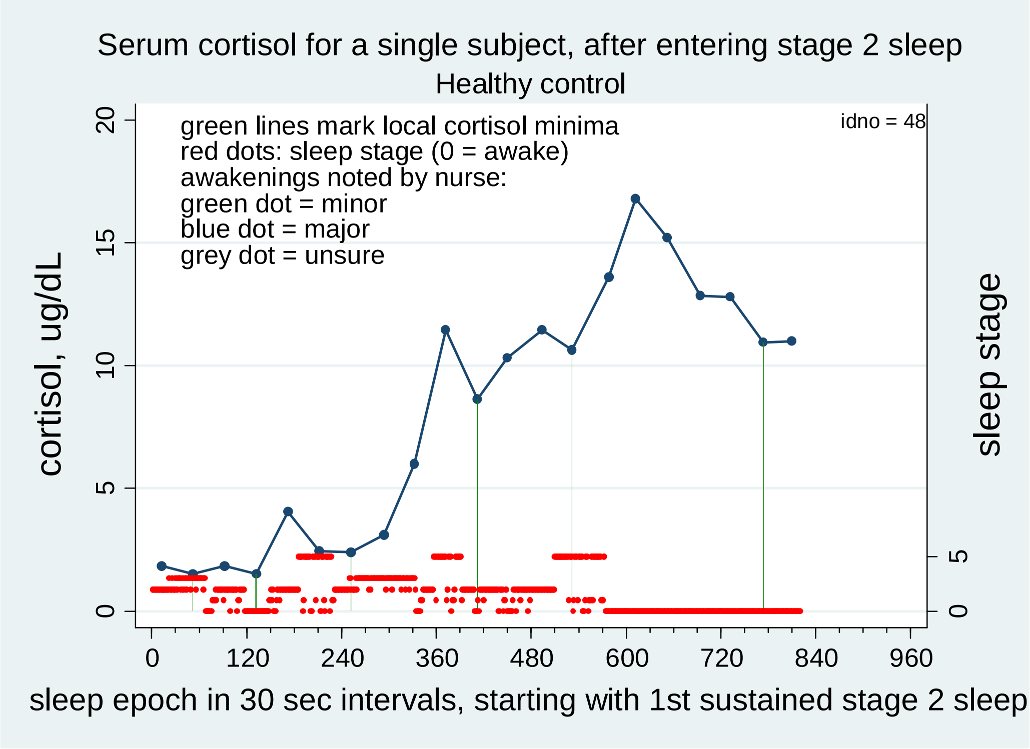

Serum cortisol for a single subject, after entering stage 2 sleep
Healthy control
green lines mark local cortisol minima
red dots: sleep stage (0 = awake)
awakenings noted by nurse:
green dot = minor
blue dot = major
grey dot = unsure
idno = 35
cortisol, ug/dL
sleep stage
0
5
10
15
20
0
5
0
120
240
360
480
600
720
840
960
sleep epoch in 30 sec intervals, starting with 1st sustained stage 2 sleep

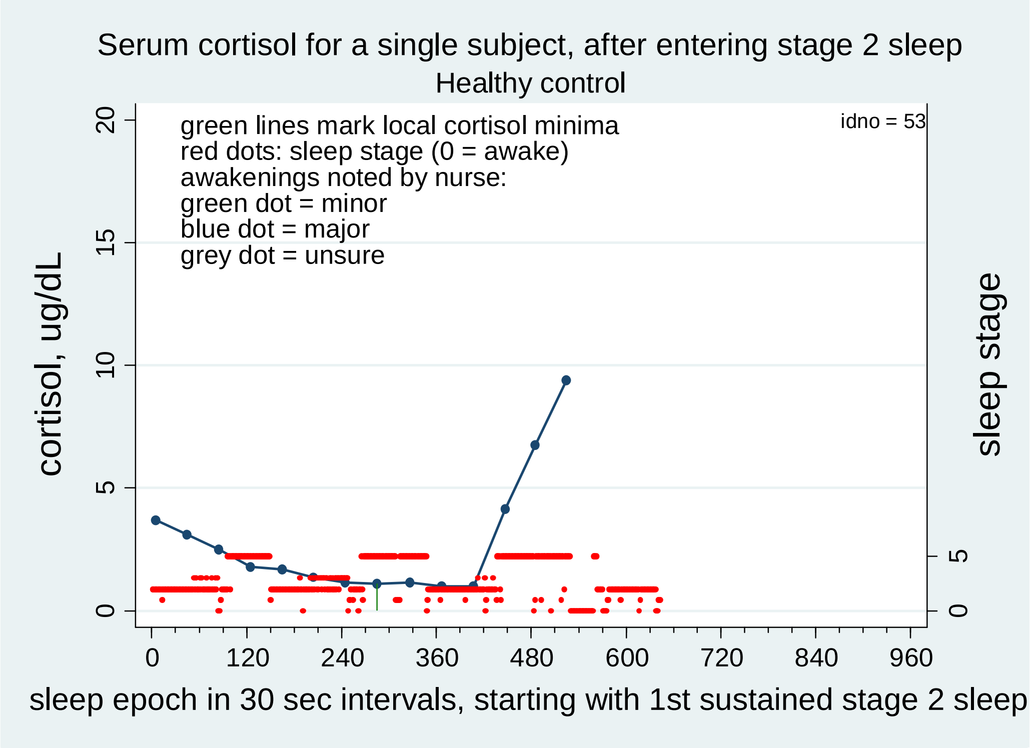

Serum cortisol for a single subject, after entering stage 2 sleep
Healthy control
green lines mark local cortisol minima
red dots: sleep stage (0 = awake)
awakenings noted by nurse:
green dot = minor
blue dot = major
grey dot = unsure
idno = 36
cortisol, ug/dL
sleep stage
0
5
10
15
20
0
5
0
120
240
360
480
600
720
840
960
sleep epoch in 30 sec intervals, starting with 1st sustained stage 2 sleep

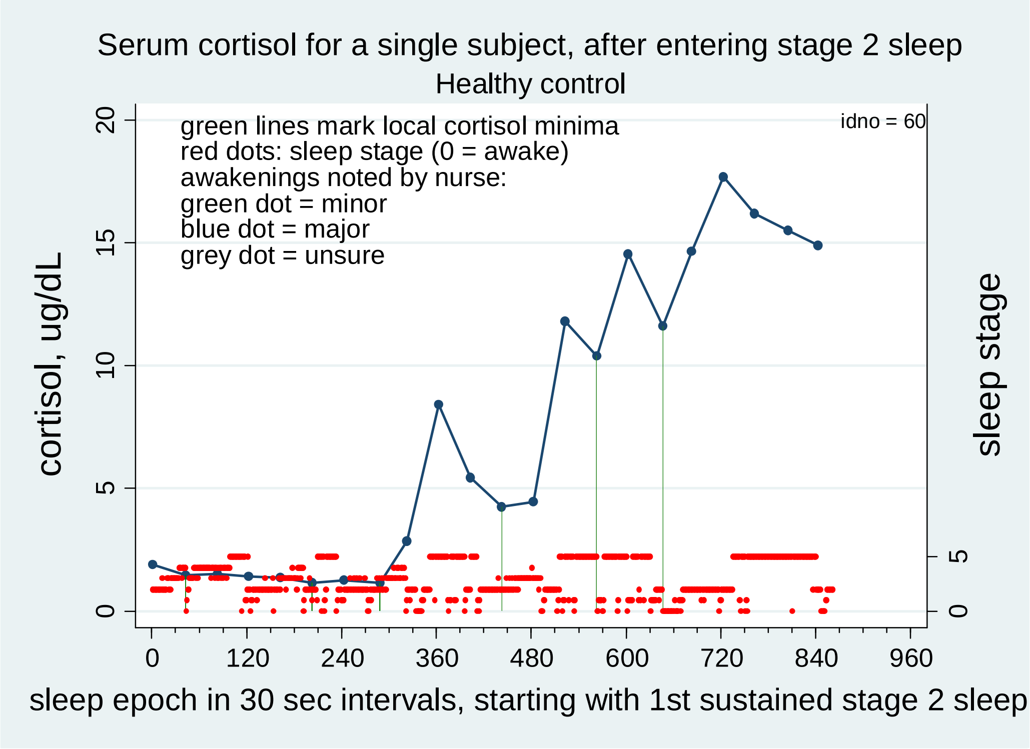

Serum cortisol for a single subject, after entering stage 2 sleep
Healthy control
green lines mark local cortisol minima
red dots: sleep stage (0 = awake)
awakenings noted by nurse:
green dot = minor
blue dot = major
grey dot = unsure
idno = 38
cortisol, ug/dL
0
5
10
15
20
sleep stage
0
5
0
120
240
360
480
600
720
840
960
sleep epoch in 30 sec intervals, starting with 1st sustained stage 2 sleep

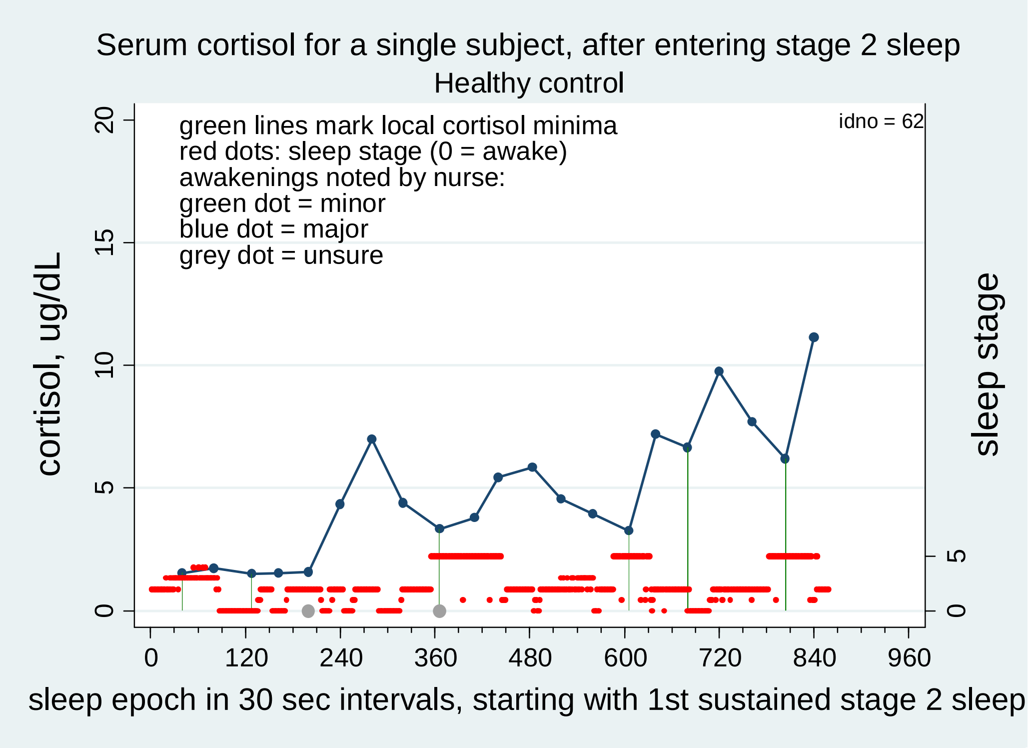

Serum cortisol for a single subject, after entering stage 2 sleep
Healthy control
green lines mark local cortisol minima
red dots: sleep stage (0 = awake)
awakenings noted by nurse:
green dot = minor
blue dot = major
grey dot = unsure
idno = 39
cortisol, ug/dL
0
5
10
15
20
sleep stage
0
5
0
120
240
360
480
600
720
840
960
sleep epoch in 30 sec intervals, starting with 1st sustained stage 2 sleep

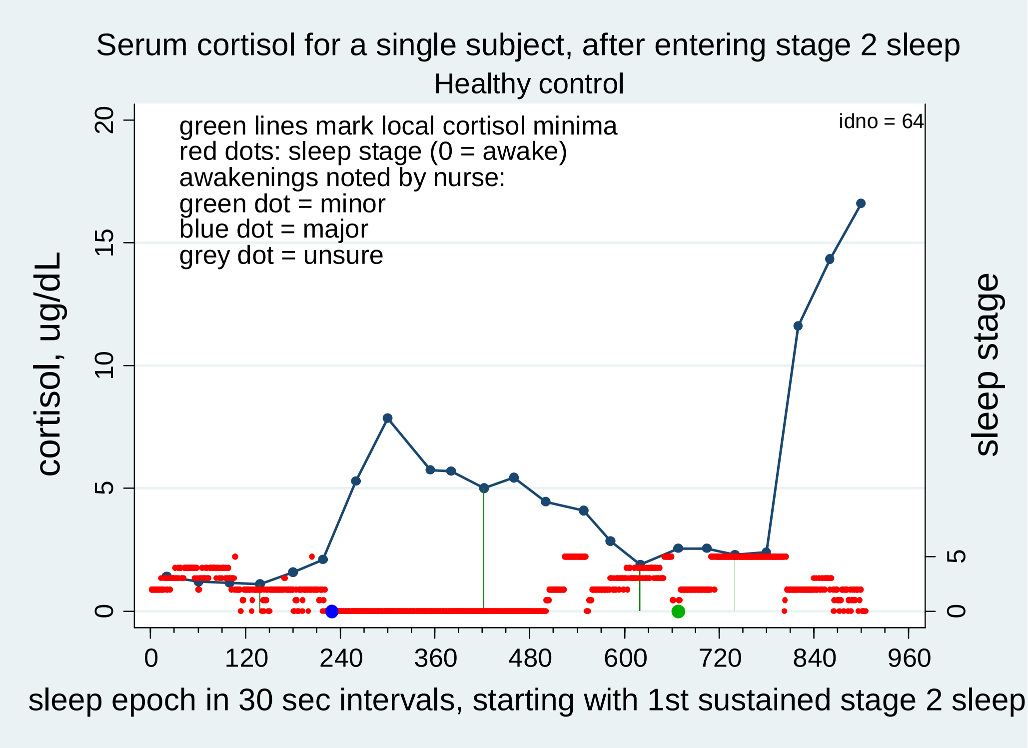

Serum cortisol for a single subject, after entering stage 2 sleep
Healthy control
green lines mark local cortisol minima
red dots: sleep stage (0 = awake)
awakenings noted by nurse:
green dot = minor
blue dot = major
grey dot = unsure
idno = 41
cortisol, ug/dL
0
5
10
15
20
sleep stage
0
5
0
120
240
360
480
600
720
840
960
sleep epoch in 30 sec intervals, starting with 1st sustained stage 2 sleep

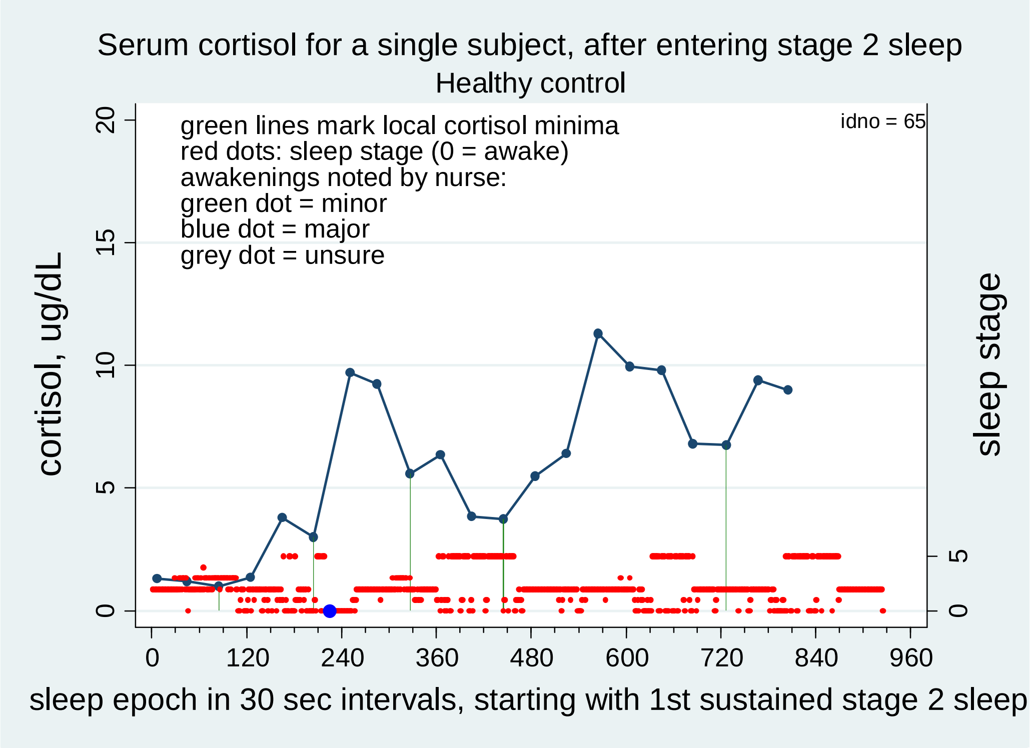

Serum cortisol for a single subject, after entering stage 2 sleep
Healthy control
green lines mark local cortisol minima
red dots: sleep stage (0 = awake)
awakenings noted by nurse:
green dot = minor
blue dot = major
grey dot = unsure
idno = 48
cortisol, ug/dL
0
5
10
15
20
sleep stage
0
5
0
120
240
360
480
600
720
840
960
sleep epoch in 30 sec intervals, starting with 1st sustained stage 2 sleep

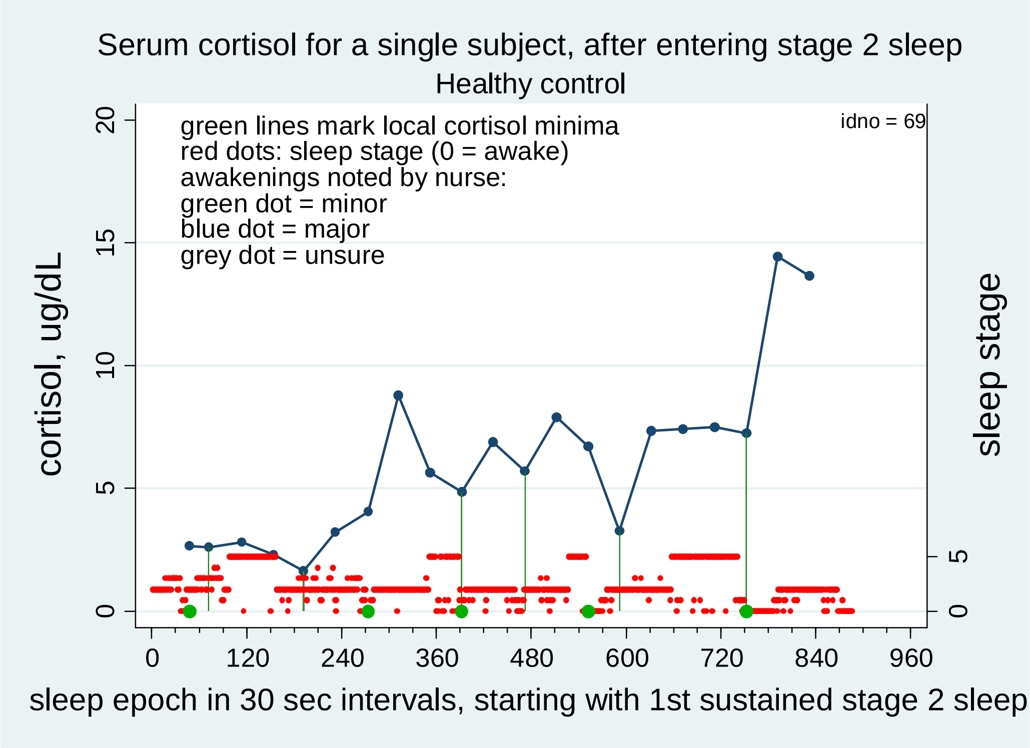

Serum cortisol for a single subject, after entering stage 2 sleep
Healthy control
green lines mark local cortisol minima
red dots: sleep stage (0 = awake)
awakenings noted by nurse:
green dot = minor
blue dot = major
grey dot = unsure
idno = 53
cortisol, ug/dL
0
5
10
15
20
sleep stage
0
5
0
120
240
360
480
600
720
840
960
sleep epoch in 30 sec intervals, starting with 1st sustained stage 2 sleep

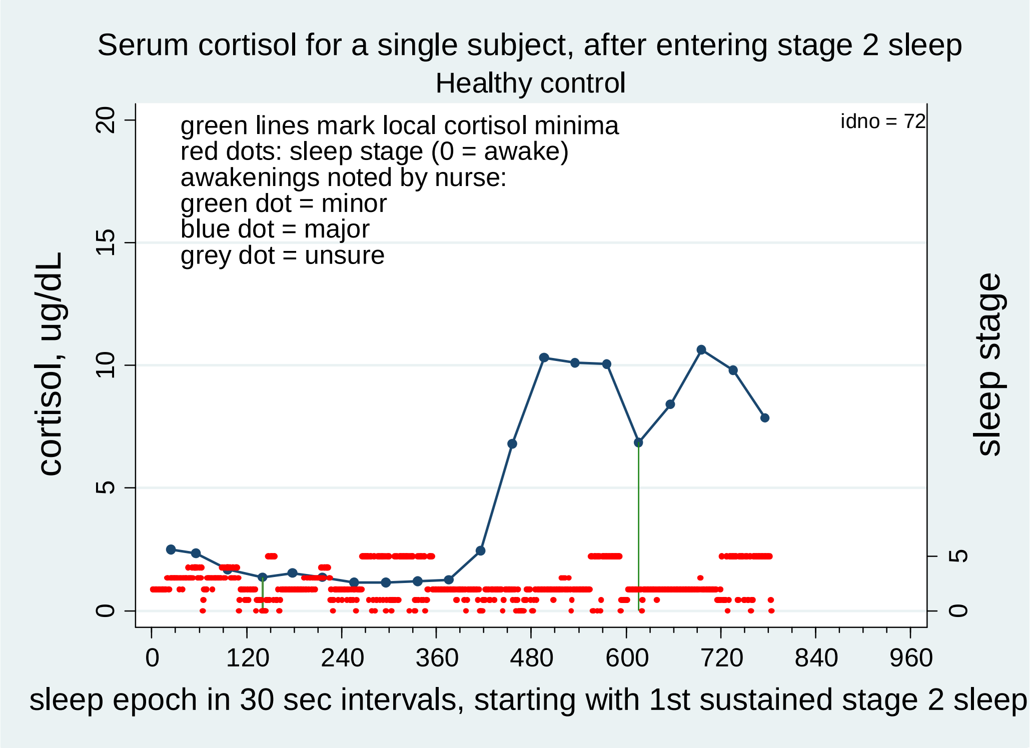

Serum cortisol for a single subject, after entering stage 2 sleep
Healthy control
green lines mark local cortisol minima
red dots: sleep stage (0 = awake)
awakenings noted by nurse:
green dot = minor
blue dot = major
grey dot = unsure
idno = 60
cortisol, ug/dL
0
5
10
15
20
sleep stage
0
5
0
120
240
360
480
600
720
840
960
sleep epoch in 30 sec intervals, starting with 1st sustained stage 2 sleep

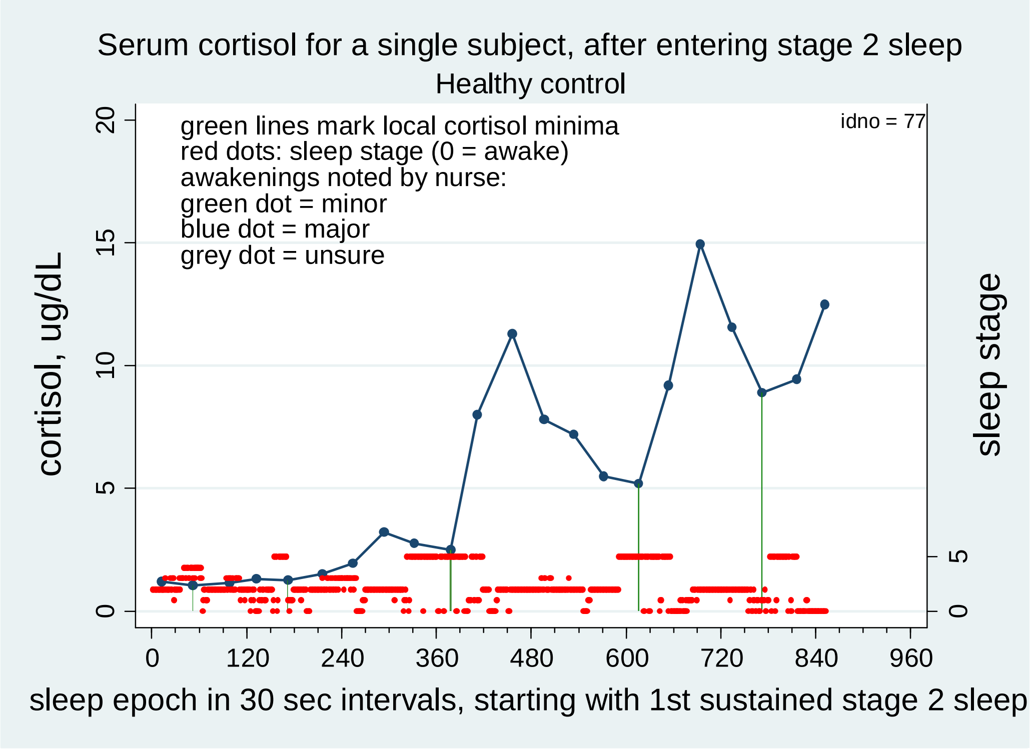

Serum cortisol for a single subject, after entering stage 2 sleep
Healthy control
green lines mark local cortisol minima
red dots: sleep stage (0 = awake)
awakenings noted by nurse:
green dot = minor
blue dot = major
grey dot = unsure
idno = 62
cortisol, ug/dL
0
5
10
15
20
sleep stage
0
5
0
120
240
360
480
600
720
840
960
sleep epoch in 30 sec intervals, starting with 1st sustained stage 2 sleep

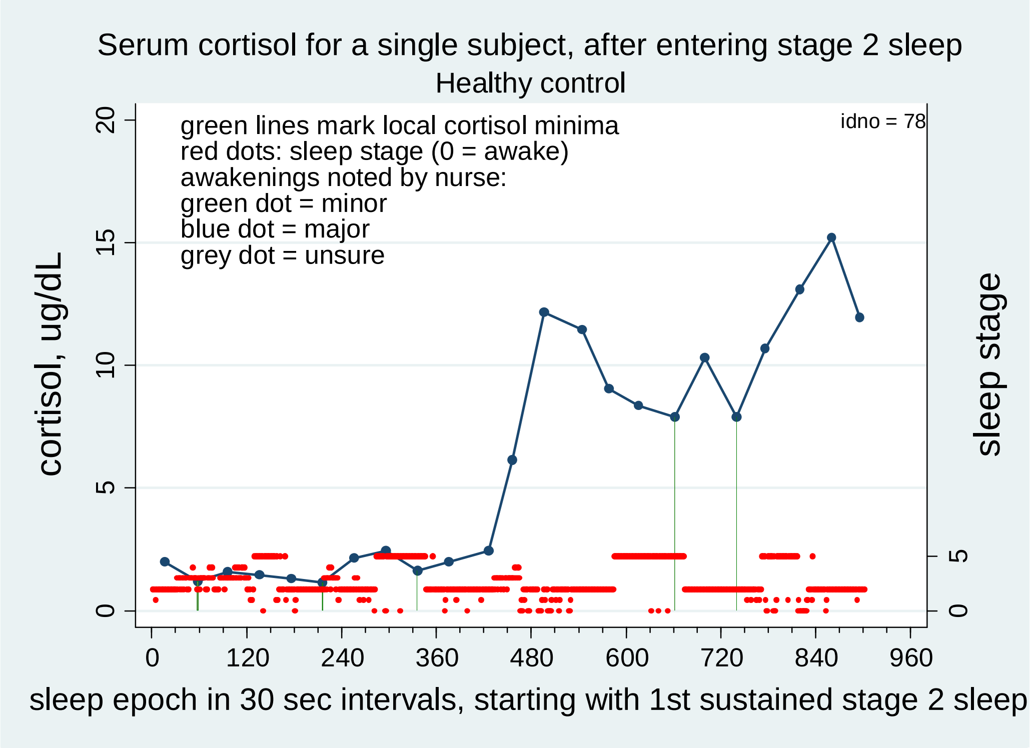

Serum cortisol for a single subject, after entering stage 2 sleep
Healthy control
green lines mark local cortisol minima
red dots: sleep stage (0 = awake)
awakenings noted by nurse:
green dot = minor
blue dot = major
grey dot = unsure
idno = 64
cortisol, ug/dL
0
5
10
15
20
sleep stage
0
5
0
120
240
360
480
600
720
840
960
sleep epoch in 30 sec intervals, starting with 1st sustained stage 2 sleep

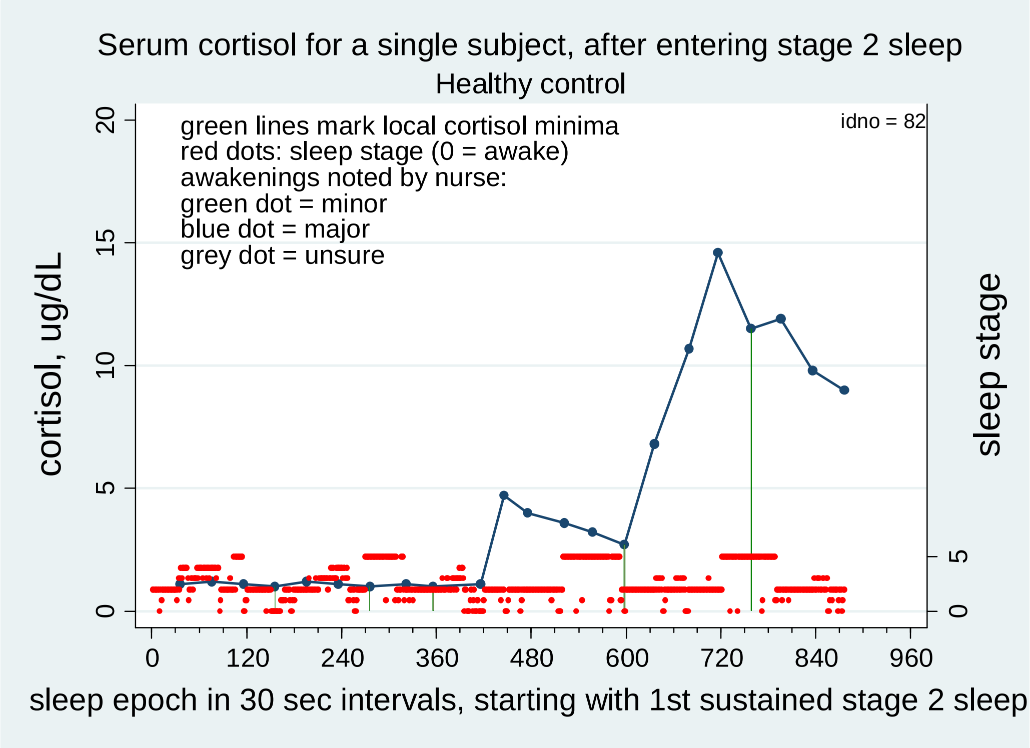

Serum cortisol for a single subject, after entering stage 2 sleep
Healthy control
green lines mark local cortisol minima
red dots: sleep stage (0 = awake)
awakenings noted by nurse:
green dot = minor
blue dot = major
grey dot = unsure
idno = 65
cortisol, ug/dL
0
5
10
15
20
sleep stage
0
5
0
120
240
360
480
600
720
840
960
sleep epoch in 30 sec intervals, starting with 1st sustained stage 2 sleep

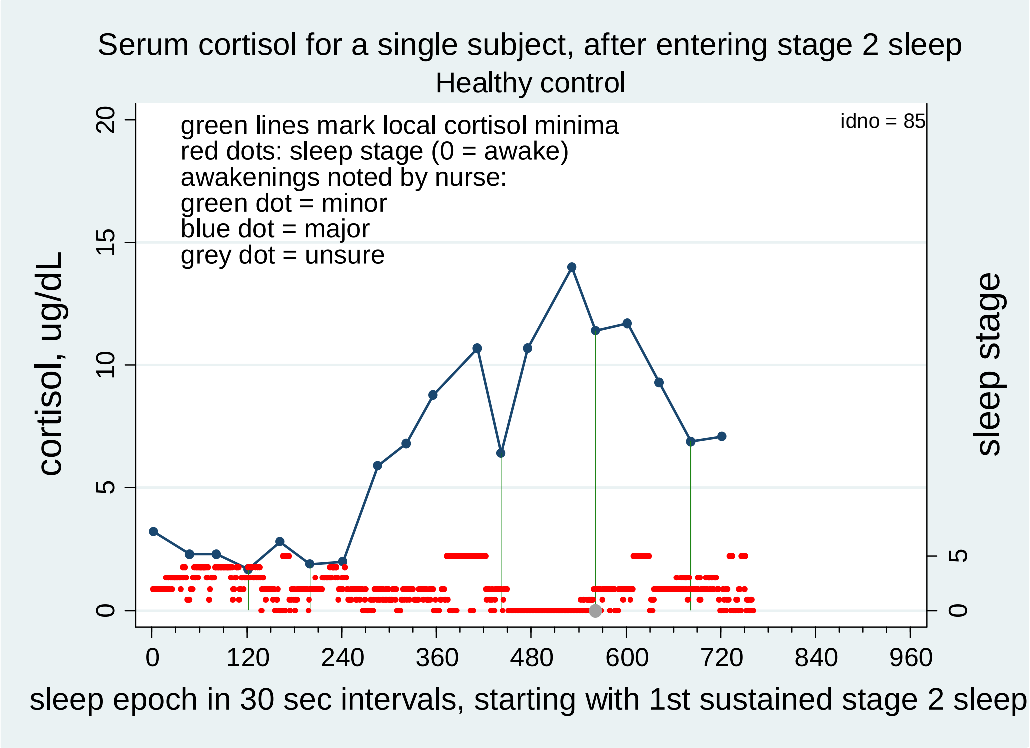

Serum cortisol for a single subject, after entering stage 2 sleep
Healthy control
green lines mark local cortisol minima
red dots: sleep stage (0 = awake)
awakenings noted by nurse:
green dot = minor
blue dot = major
grey dot = unsure
idno = 69
cortisol, ug/dL
0
5
10
15
20
sleep stage
0
5
0
120
240
360
480
600
720
840
960
sleep epoch in 30 sec intervals, starting with 1st sustained stage 2 sleep

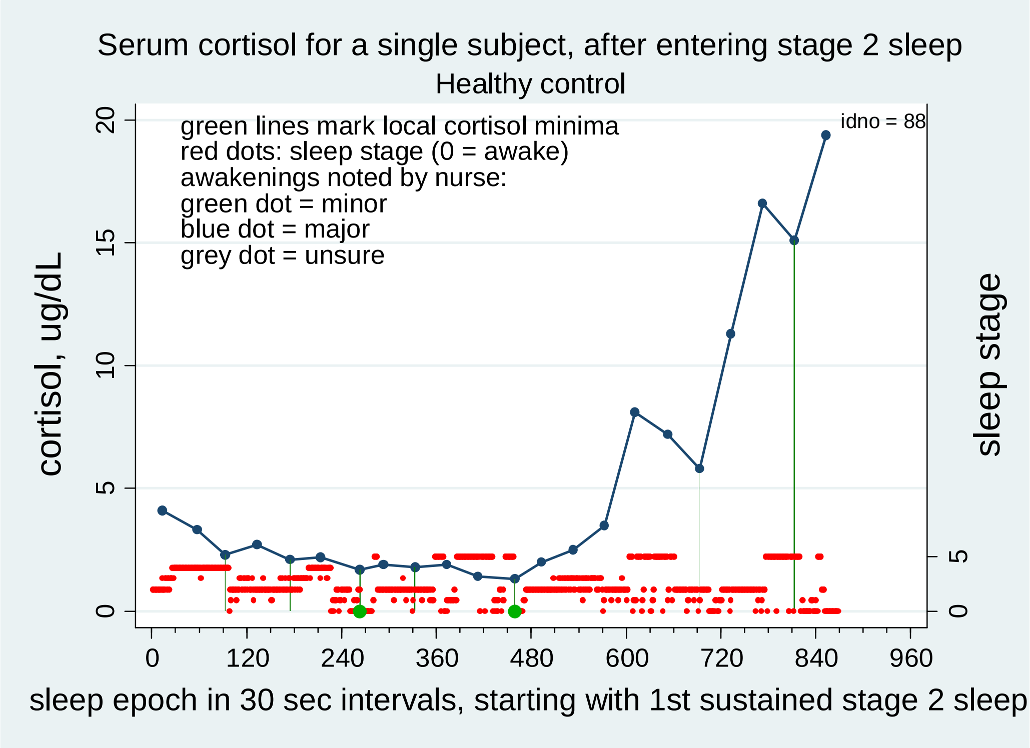

Serum cortisol for a single subject, after entering stage 2 sleep
Healthy control
green lines mark local cortisol minima
red dots: sleep stage (0 = awake)
awakenings noted by nurse:
green dot = minor
blue dot = major
grey dot = unsure
idno = 72
cortisol, ug/dL
0
5
10
15
20
sleep stage
0
5
0
120
240
360
480
600
720
840
960
sleep epoch in 30 sec intervals, starting with 1st sustained stage 2 sleep


Serum cortisol for a single subject, after entering stage 2 sleep
Healthy control
green lines mark local cortisol minima
red dots: sleep stage (0 = awake)
awakenings noted by nurse:
green dot = minor
blue dot = major
grey dot = unsure
idno = 77
cortisol, ug/dL
0
5
10
15
20
sleep stage
0
5
0
120
240
360
480
600
720
840
960
sleep epoch in 30 sec intervals, starting with 1st sustained stage 2 sleep

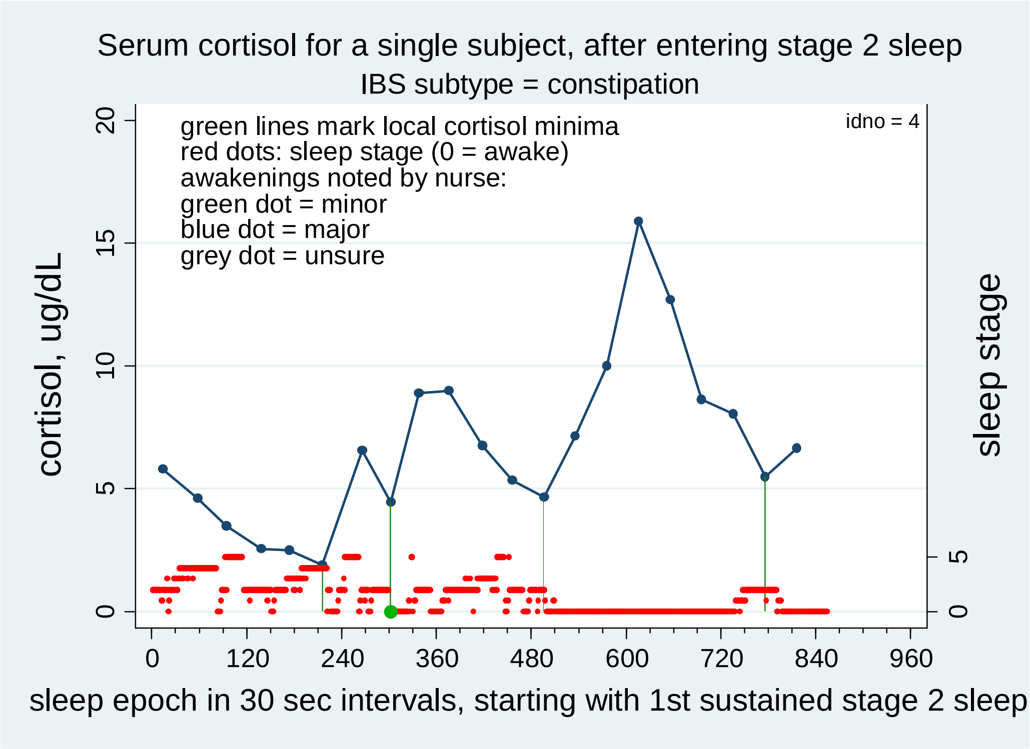

Serum cortisol for a single subject, after entering stage 2 sleep
Healthy control
green lines mark local cortisol minima
red dots: sleep stage (0 = awake)
awakenings noted by nurse:
green dot = minor
blue dot = major
grey dot = unsure
idno = 78
cortisol, ug/dL
0
5
10
15
20
sleep stage
0
5
0
120
240
360
480
600
720
840
960
sleep epoch in 30 sec intervals, starting with 1st sustained stage 2 sleep

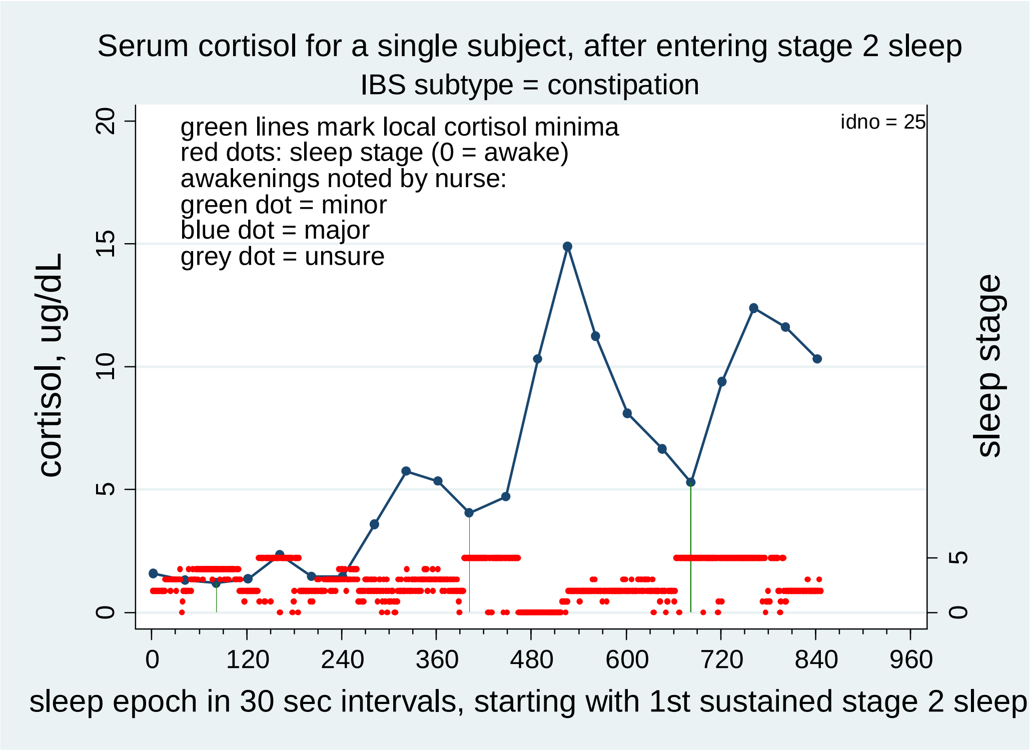

Serum cortisol for a single subject, after entering stage 2 sleep
Healthy control
green lines mark local cortisol minima
red dots: sleep stage (0 = awake)
awakenings noted by nurse:
green dot = minor
blue dot = major
grey dot = unsure
idno = 82
cortisol, ug/dL
0
5
10
15
20
sleep stage
0
5
0
120
240
360
480
600
720
840
960
sleep epoch in 30 sec intervals, starting with 1st sustained stage 2 sleep

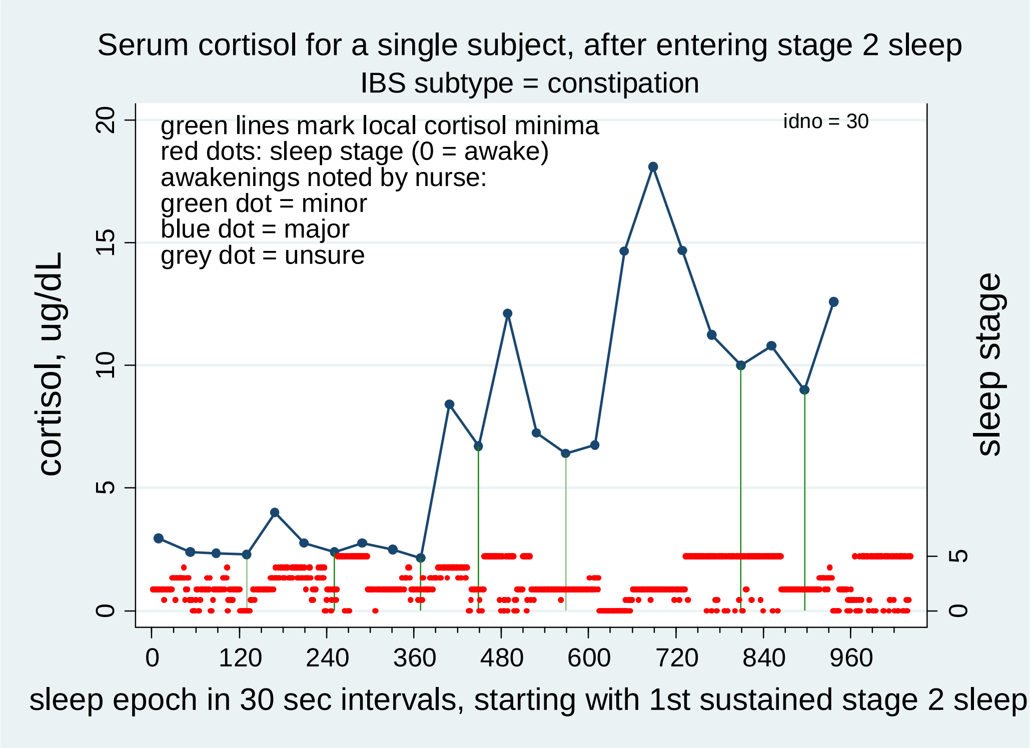

Serum cortisol for a single subject, after entering stage 2 sleep
Healthy control
green lines mark local cortisol minima
red dots: sleep stage (0 = awake)
awakenings noted by nurse:
green dot = minor
blue dot = major
grey dot = unsure
idno = 85
cortisol, ug/dL
0
5
10
15
20
sleep stage
0
5
0
120
240
360
480
600
720
840
960
sleep epoch in 30 sec intervals, starting with 1st sustained stage 2 sleep

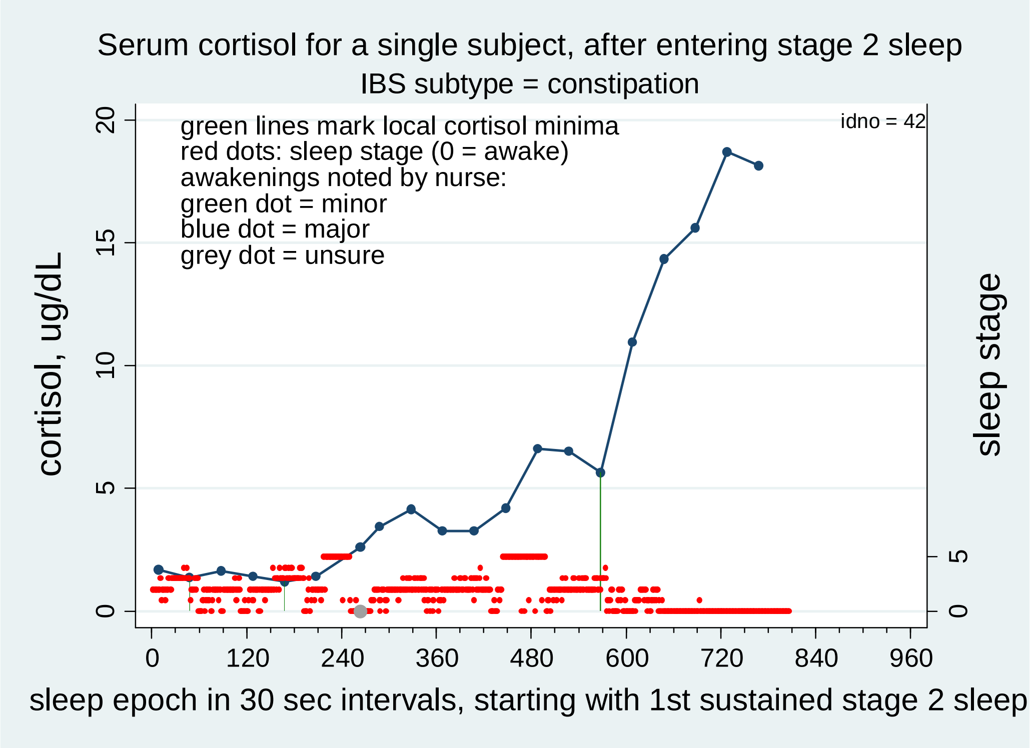

Serum cortisol for a single subject, after entering stage 2 sleep
Healthy control
green lines mark local cortisol minima
red dots: sleep stage (0 = awake)
awakenings noted by nurse:
green dot = minor
blue dot = major
grey dot = unsure
idno = 88
cortisol, ug/dL
0
5
10
15
20
sleep stage
0
5
0
120
240
360
480
600
720
840
960
sleep epoch in 30 sec intervals, starting with 1st sustained stage 2 sleep

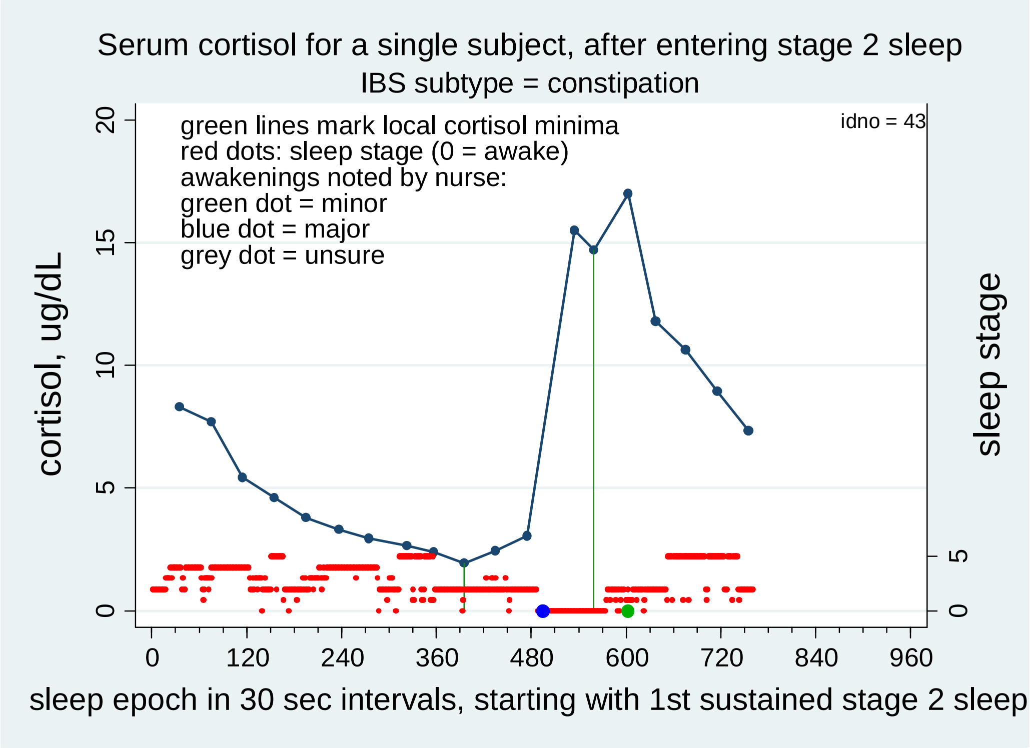
Constipation-Predominant

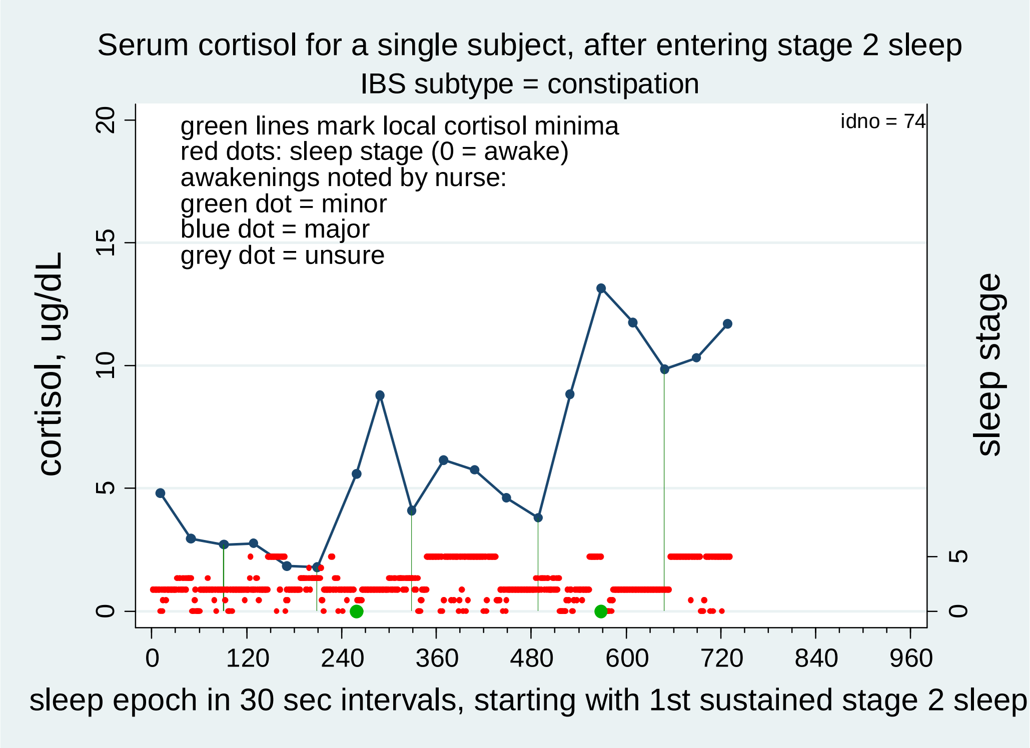


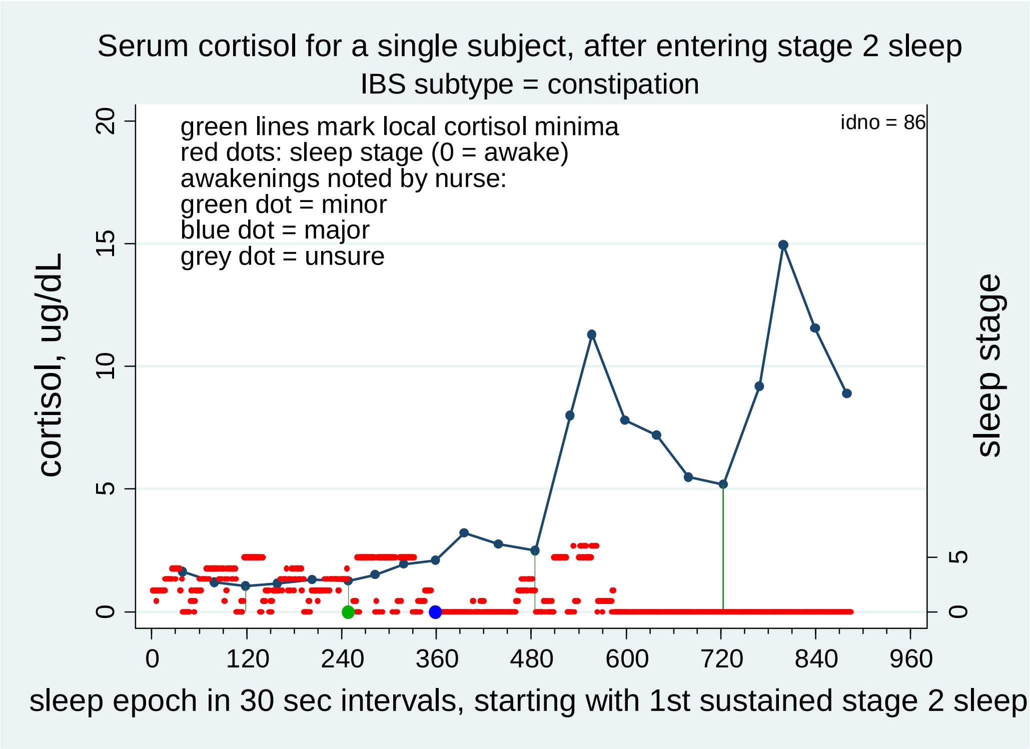



Serum cortisol for a single subject, after entering stage 2 sleep
IBS subtype = constipation
green lines mark local cortisol minima
red dots: sleep stage (0 = awake)
awakenings noted by nurse:
green dot = minor
blue dot = major
grey dot = unsure
idno = 30
cortisol, ug/dL
0
5
10
15
20
sleep stage
0
5
0
120
240
360
480
600
720
840
960
sleep epoch in 30 sec intervals, starting with 1st sustained stage 2 sleep

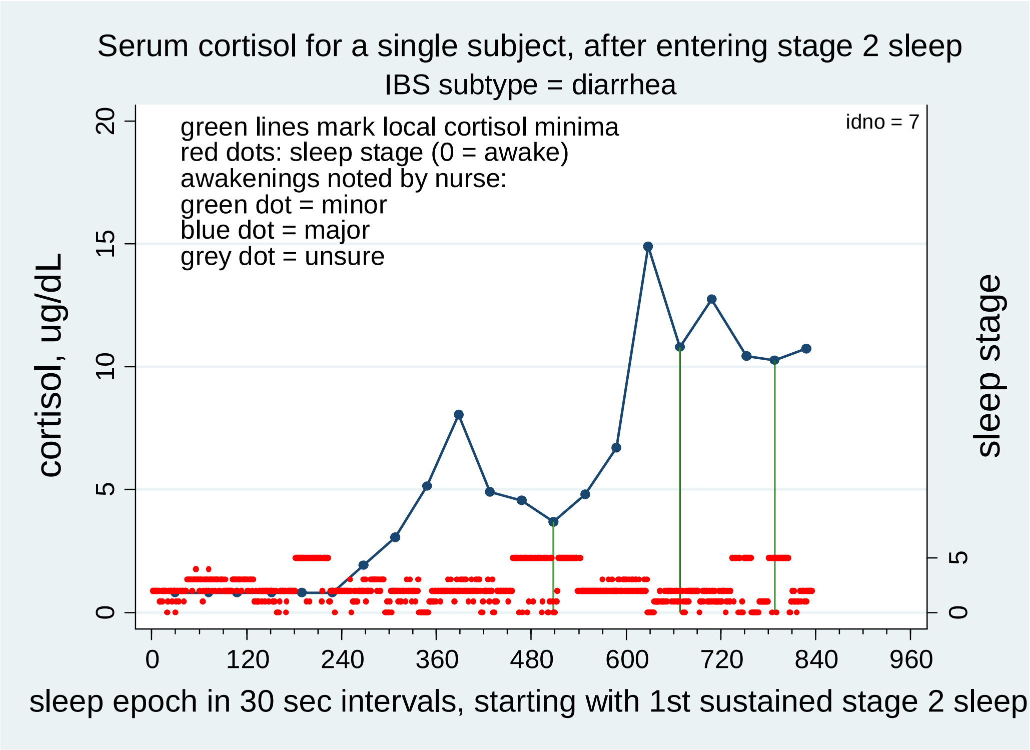

Serum cortisol for a single subject, after entering stage 2 sleep
IBS subtype = constipation
green lines mark local cortisol minima
red dots: sleep stage (0 = awake)
awakenings noted by nurse:
green dot = minor
blue dot = major
grey dot = unsure
idno = 42
cortisol, ug/dL
0
5
10
15
20
sleep stage
0
5
0
120
240
360
480
600
720
840
960
sleep epoch in 30 sec intervals, starting with 1st sustained stage 2 sleep

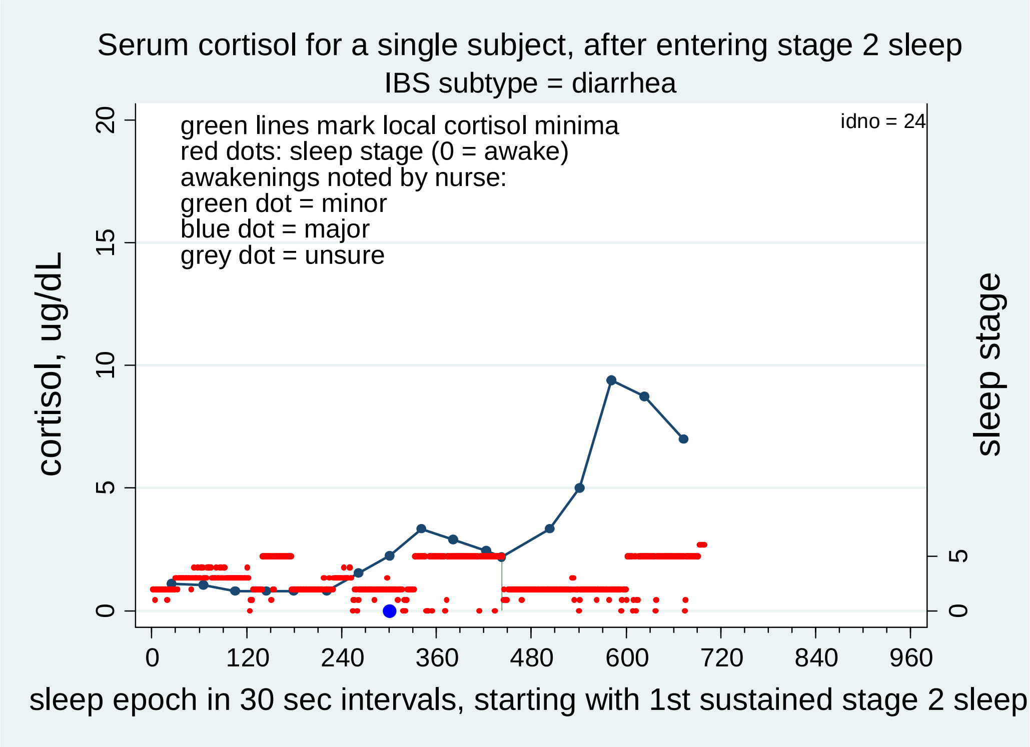

Serum cortisol for a single subject, after entering stage 2 sleep
IBS subtype = constipation
green lines mark local cortisol minima
red dots: sleep stage (0 = awake)
awakenings noted by nurse:
green dot = minor
blue dot = major
grey dot = unsure
idno = 43
cortisol, ug/dL
0
5
10
15
20
sleep stage
0
5
0
120
240
360
480
600
720
840
960
sleep epoch in 30 sec intervals, starting with 1st sustained stage 2 sleep

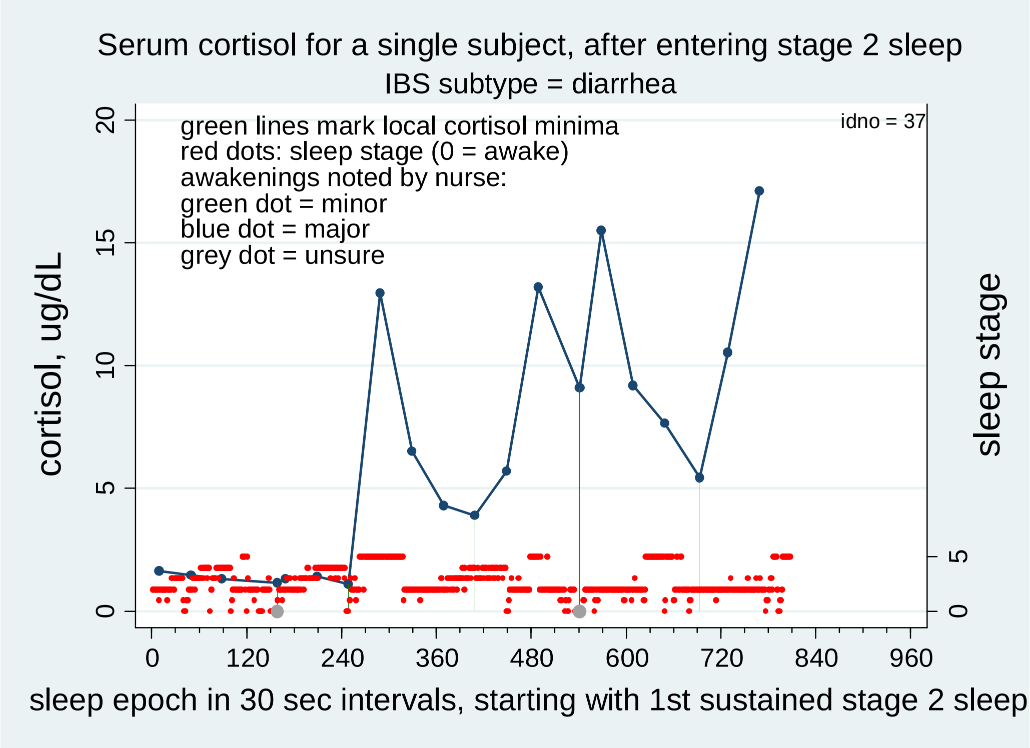

Serum cortisol for a single subject, after entering stage 2 sleep
IBS subtype = constipation
green lines mark local cortisol minima
red dots: sleep stage (0 = awake)
awakenings noted by nurse:
green dot = minor
blue dot = major
grey dot = unsure
idno = 74
cortisol, ug/dL
0
5
10
15
20
sleep stage
0
5
0
120
240
360
480
600
720
840
960
sleep epoch in 30 sec intervals, starting with 1st sustained stage 2 sleep

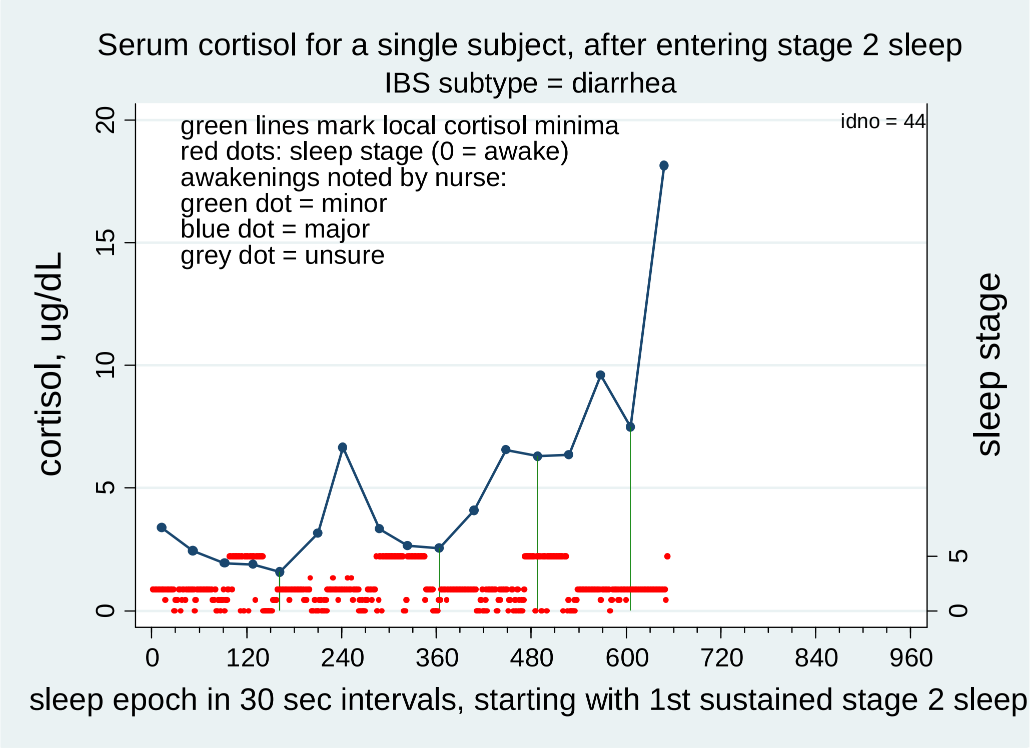


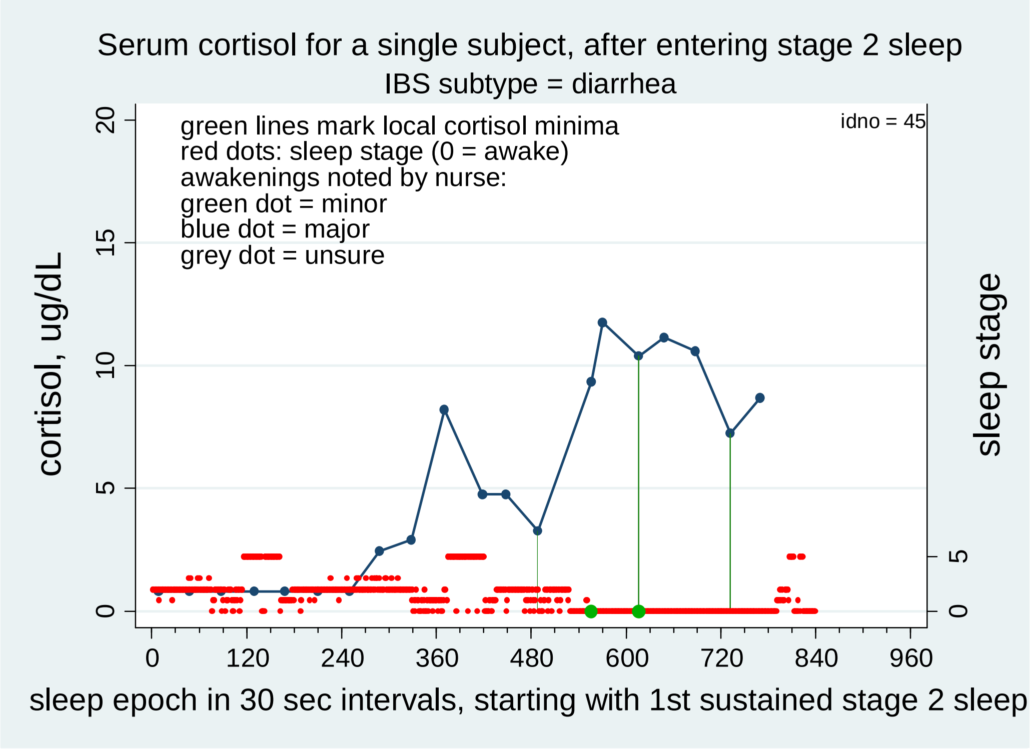
<u>Diarrhea-Predominant</u>

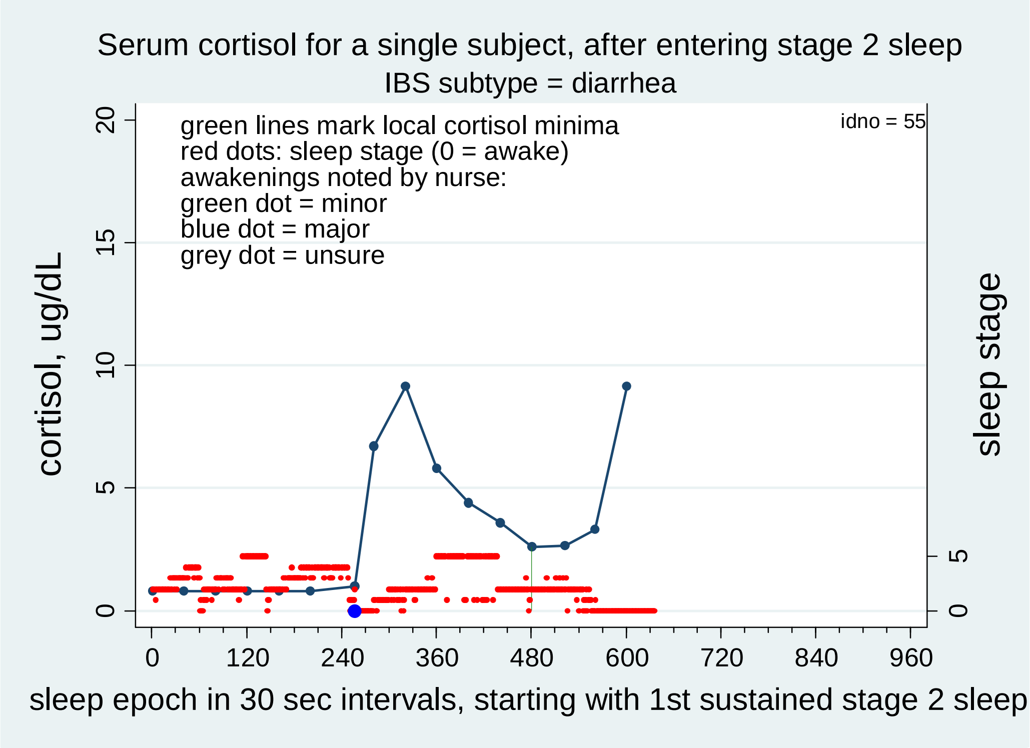


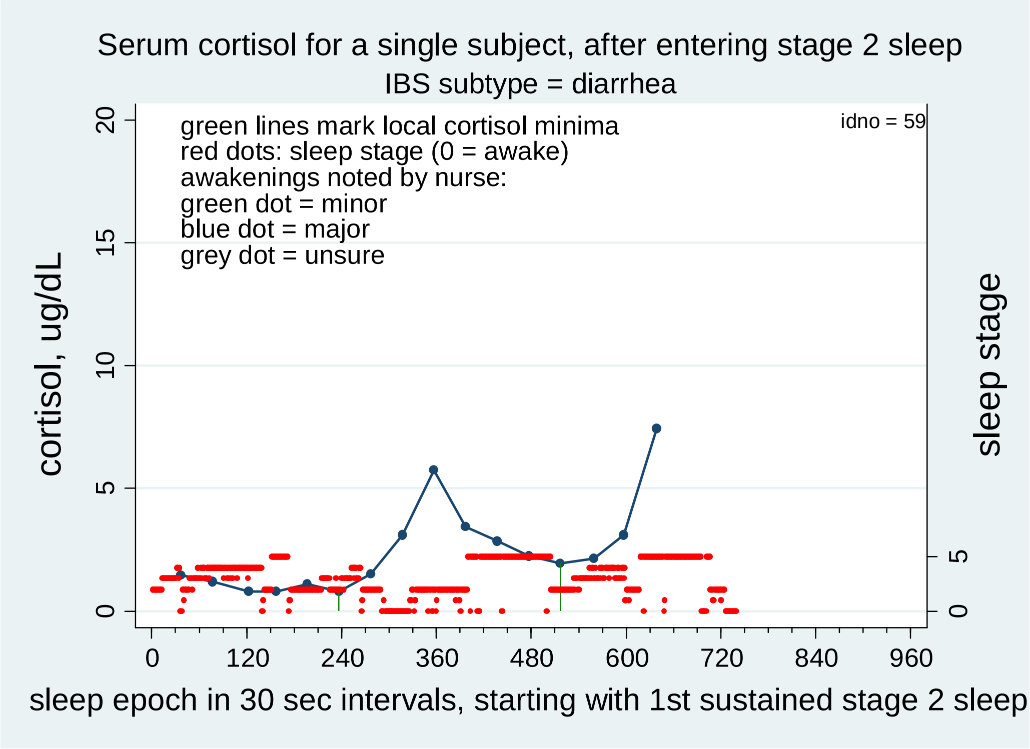

Serum cortisol for a single subject, after entering stage 2 sleep
IBS subtype = diarrhea
green lines mark local cortisol minima
red dots: sleep stage (0 = awake)
awakenings noted by nurse:
green dot = minor
blue dot = major
grey dot = unsure
idno = 24
cortisol, ug/dL
sleep stage
0
5
10
15
20
0
5
0
120
240
360
480
600
720
840
960
sleep epoch in 30 sec intervals, starting with 1st sustained stage 2 sleep

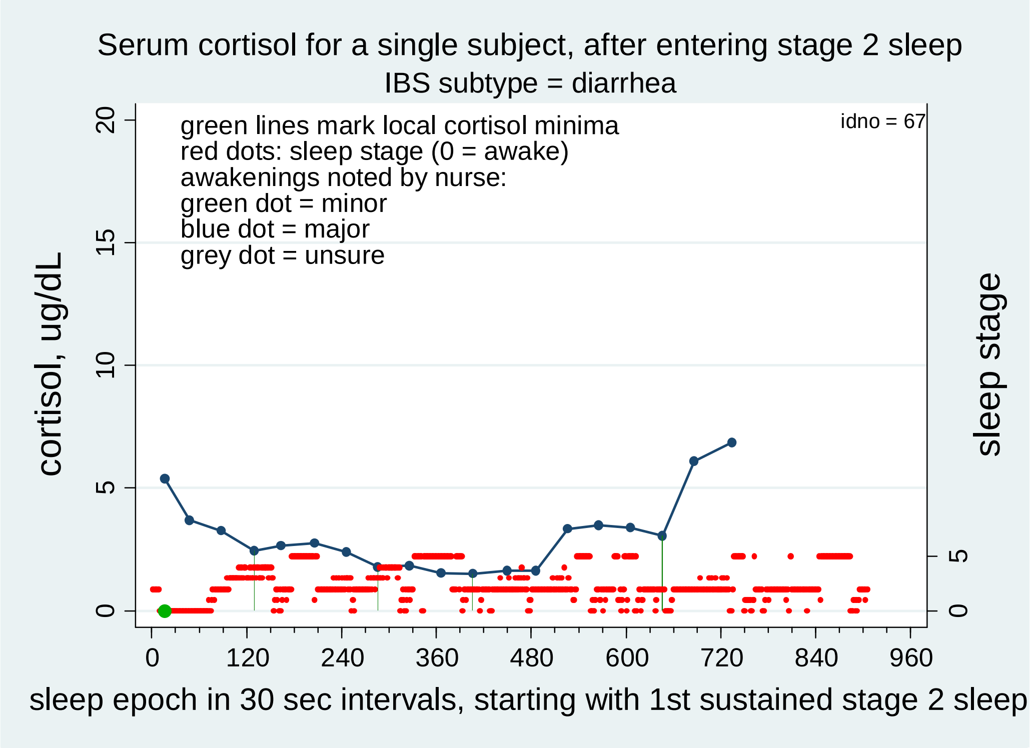

Serum cortisol for a single subject, after entering stage 2 sleep
IBS subtype = diarrhea
green lines mark local cortisol minima
red dots: sleep stage (0 = awake)
awakenings noted by nurse:
green dot = minor
blue dot = major
grey dot = unsure
idno = 37
cortisol, ug/dL
sleep stage
0
5
10
15
20
0
5
0
120
240
360
480
600
720
840
960
sleep epoch in 30 sec intervals, starting with 1st sustained stage 2 sleep

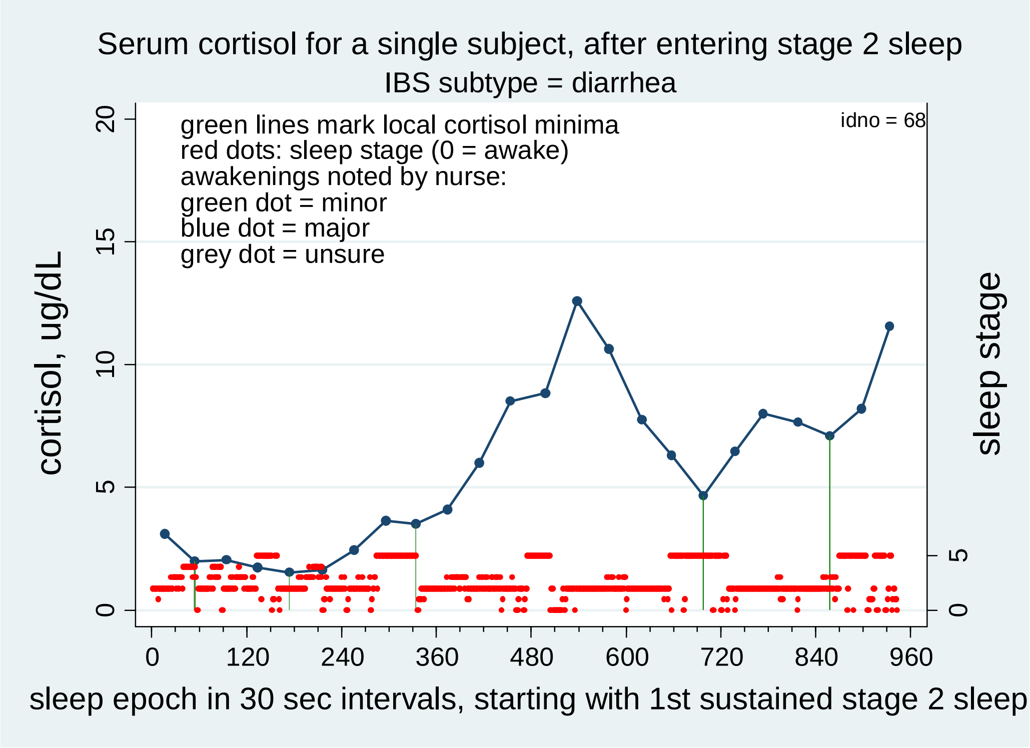

Serum cortisol for a single subject, after entering stage 2 sleep
IBS subtype = diarrhea
green lines mark local cortisol minima
red dots: sleep stage (0 = awake)
awakenings noted by nurse:
green dot = minor
blue dot = major
grey dot = unsure
idno = 44
cortisol, ug/dL
0
5
10
15
20
sleep stage
0
5
0
120
240
360
480
600
720
840
960
sleep epoch in 30 sec intervals, starting with 1st sustained stage 2 sleep

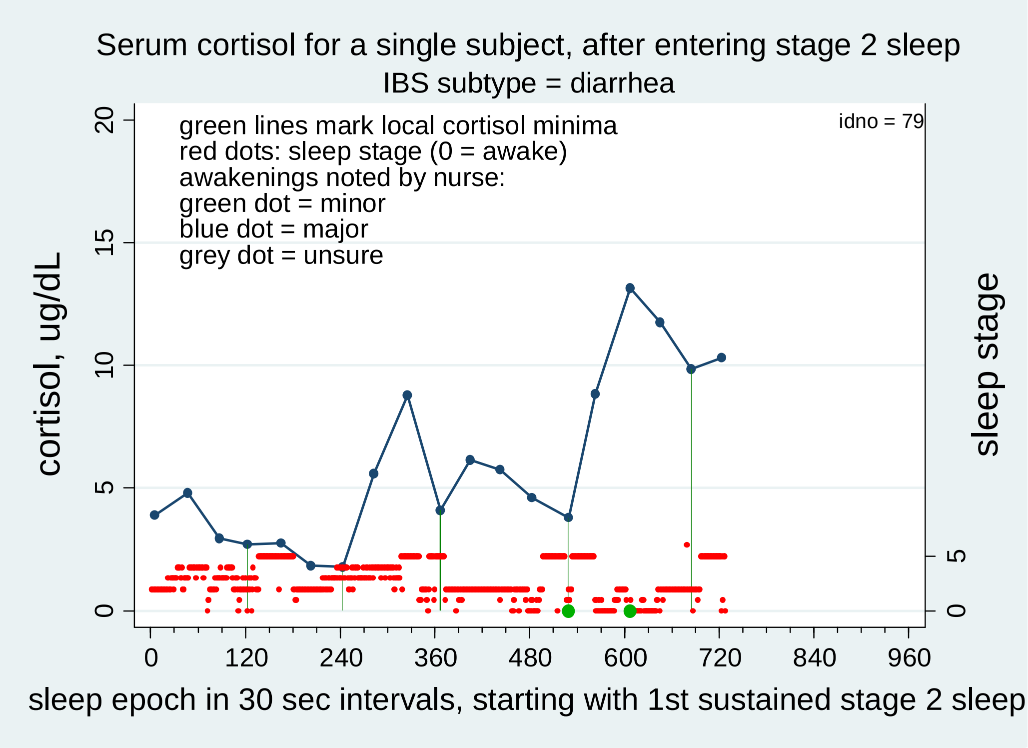

Serum cortisol for a single subject, after entering stage 2 sleep
IBS subtype = diarrhea
green lines mark local cortisol minima
red dots: sleep stage (0 = awake)
awakenings noted by nurse:
green dot = minor
blue dot = major
grey dot = unsure
idno = 45
cortisol, ug/dL
0
5
10
15
20
sleep stage
0
5
0
120
240
360
480
600
720
840
960
sleep epoch in 30 sec intervals, starting with 1st sustained stage 2 sleep


Serum cortisol for a single subject, after entering stage 2 sleep
IBS subtype = diarrhea
green lines mark local cortisol minima
red dots: sleep stage (0 = awake)
awakenings noted by nurse:
green dot = minor
blue dot = major
grey dot = unsure
idno = 55
cortisol, ug/dL
0
5
10
15
20
sleep stage
0
5
0
120
240
360
480
600
720
840
960
sleep epoch in 30 sec intervals, starting with 1st sustained stage 2 sleep

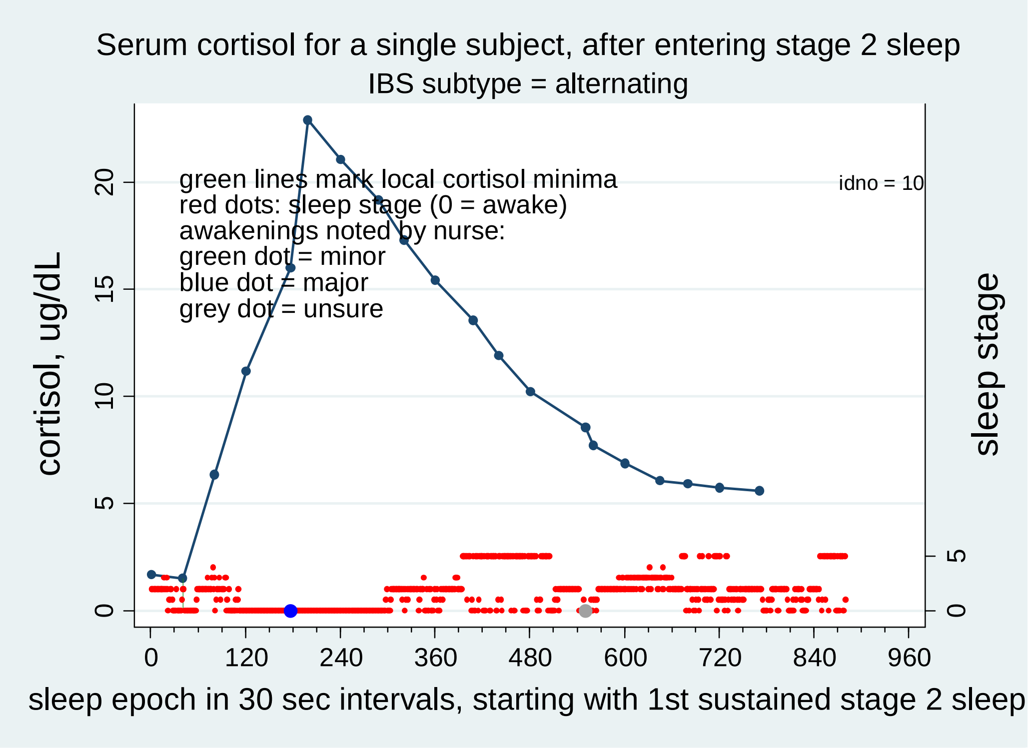

Serum cortisol for a single subject, after entering stage 2 sleep
IBS subtype = diarrhea
green lines mark local cortisol minima
red dots: sleep stage (0 = awake)
awakenings noted by nurse:
green dot = minor
blue dot = major
grey dot = unsure
idno = 59
cortisol, ug/dL
0
5
10
15
20
sleep stage
0
5
0
120
240
360
480
600
720
840
960
sleep epoch in 30 sec intervals, starting with 1st sustained stage 2 sleep

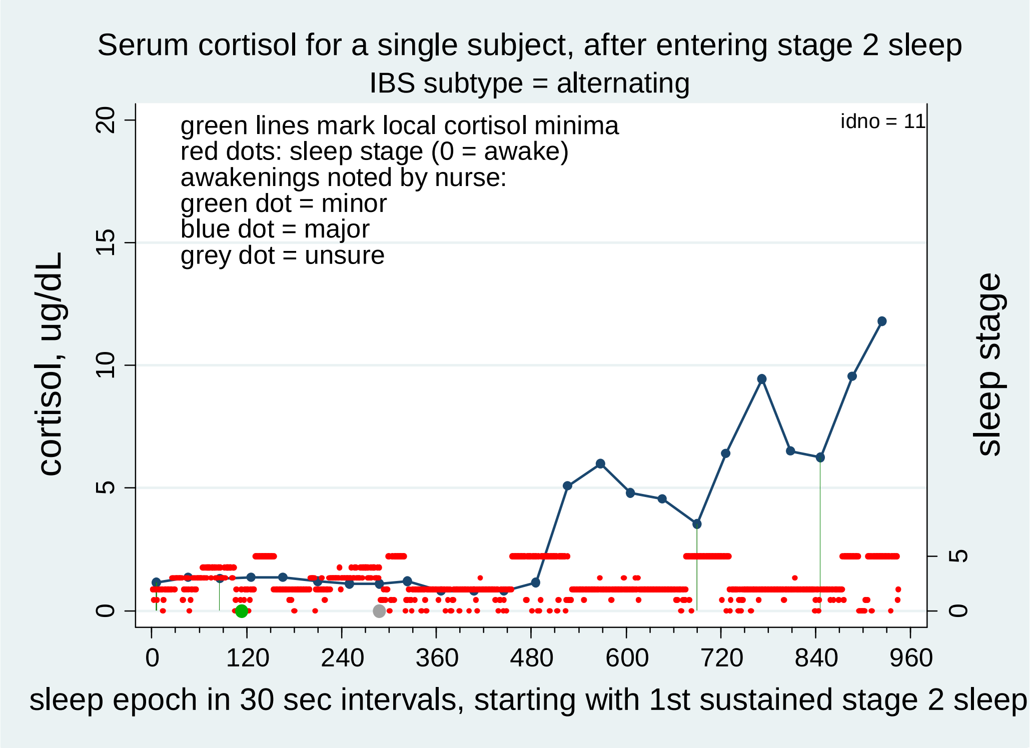

Serum cortisol for a single subject, after entering stage 2 sleep
IBS subtype = diarrhea
green lines mark local cortisol minima
red dots: sleep stage (0 = awake)
awakenings noted by nurse:
green dot = minor
blue dot = major
grey dot = unsure
idno = 67
cortisol, ug/dL
0
5
10
15
20
sleep stage
0
5
0
120
240
360
480
600
720
840
960
sleep epoch in 30 sec intervals, starting with 1st sustained stage 2 sleep

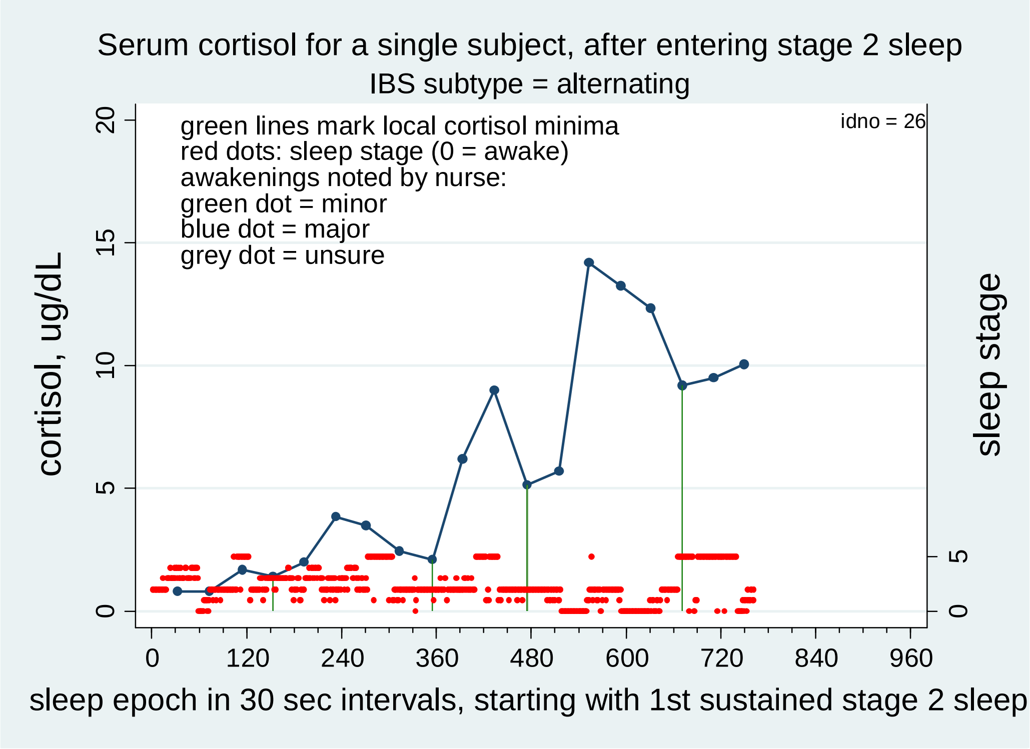

Serum cortisol for a single subject, after entering stage 2 sleep
IBS subtype = diarrhea
green lines mark local cortisol minima
red dots: sleep stage (0 = awake)
awakenings noted by nurse:
green dot = minor
blue dot = major
grey dot = unsure
idno = 68
cortisol, ug/dL
0
5
10
15
20
sleep stage
0
5
0
120
240
360
480
600
720
840
960
sleep epoch in 30 sec intervals, starting with 1st sustained stage 2 sleep

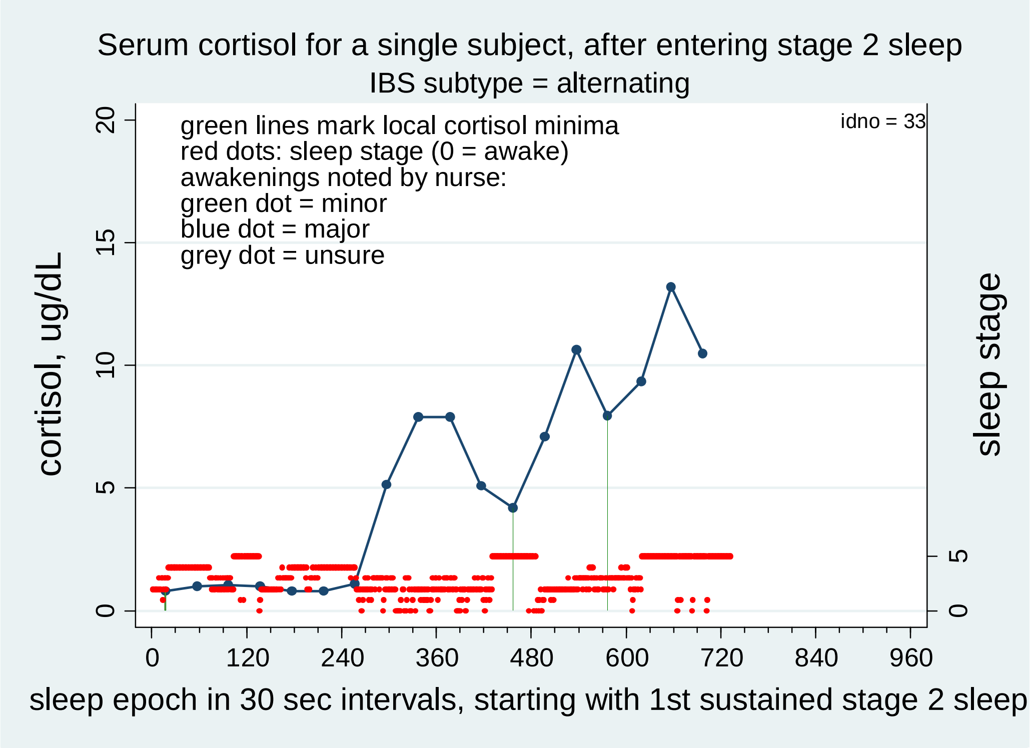


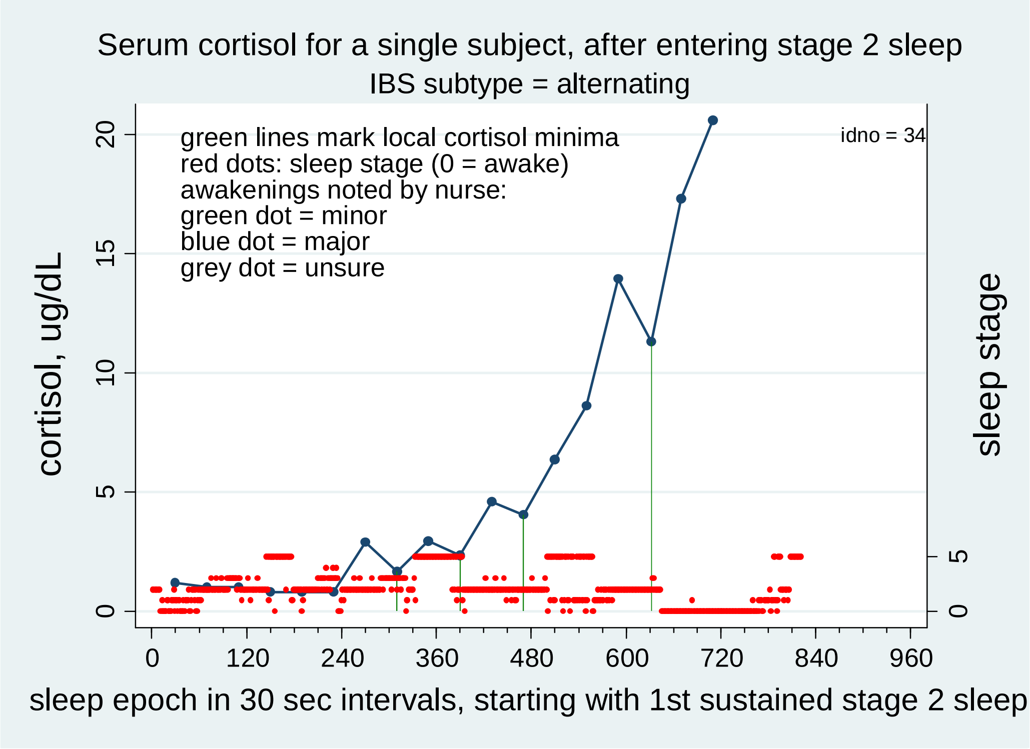
<u>Alternating</u>

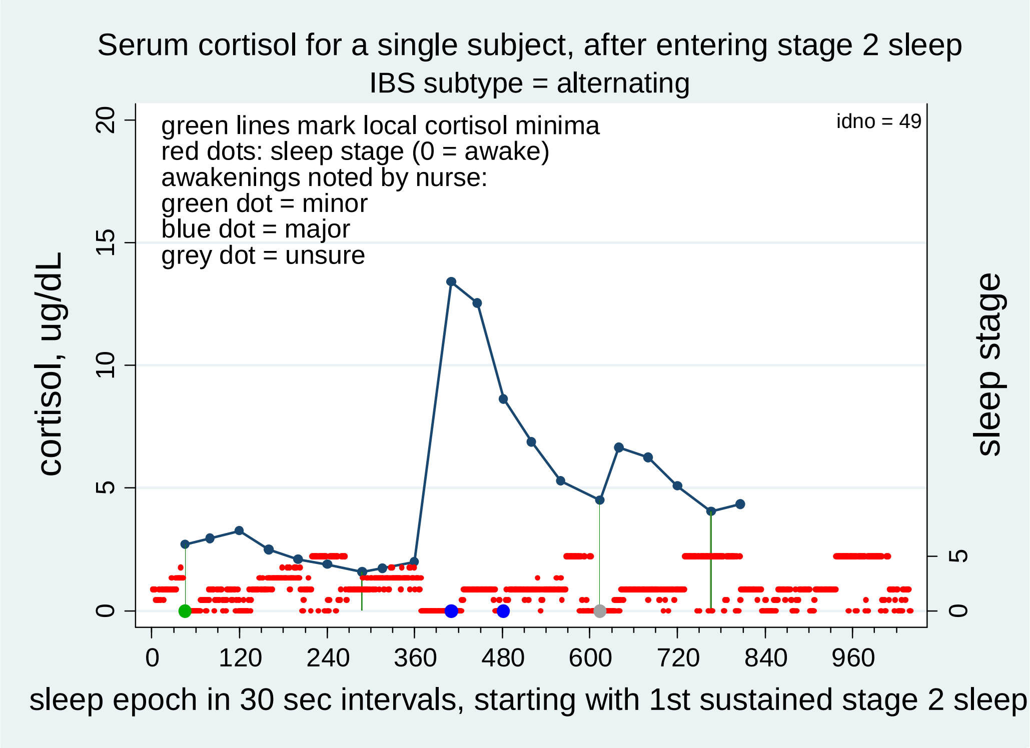


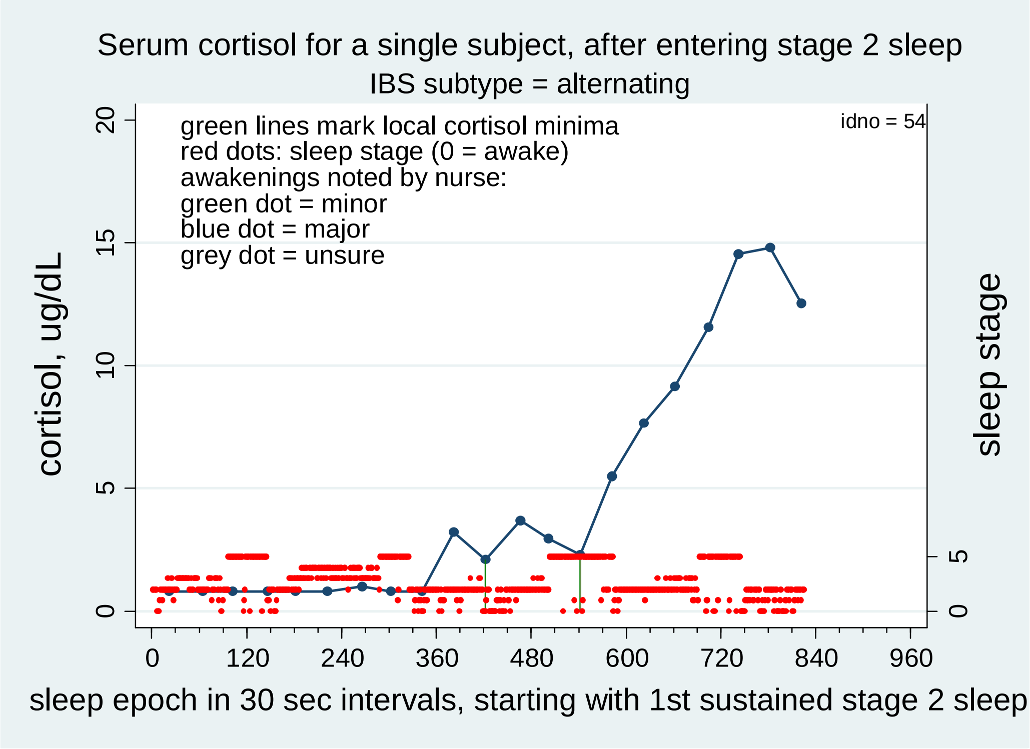

Serum cortisol for a single subject, after entering stage 2 sleep
IBS subtype = alternating
green lines mark local cortisol minima
red dots: sleep stage (0 = awake)
awakenings noted by nurse:
green dot = minor
blue dot = major
grey dot = unsure
idno = 11
cortisol, ug/dL
0
5
10
15
20
sleep stage
0
5
0
120
240
360
480
600
720
840
960
sleep epoch in 30 sec intervals, starting with 1st sustained stage 2 sleep

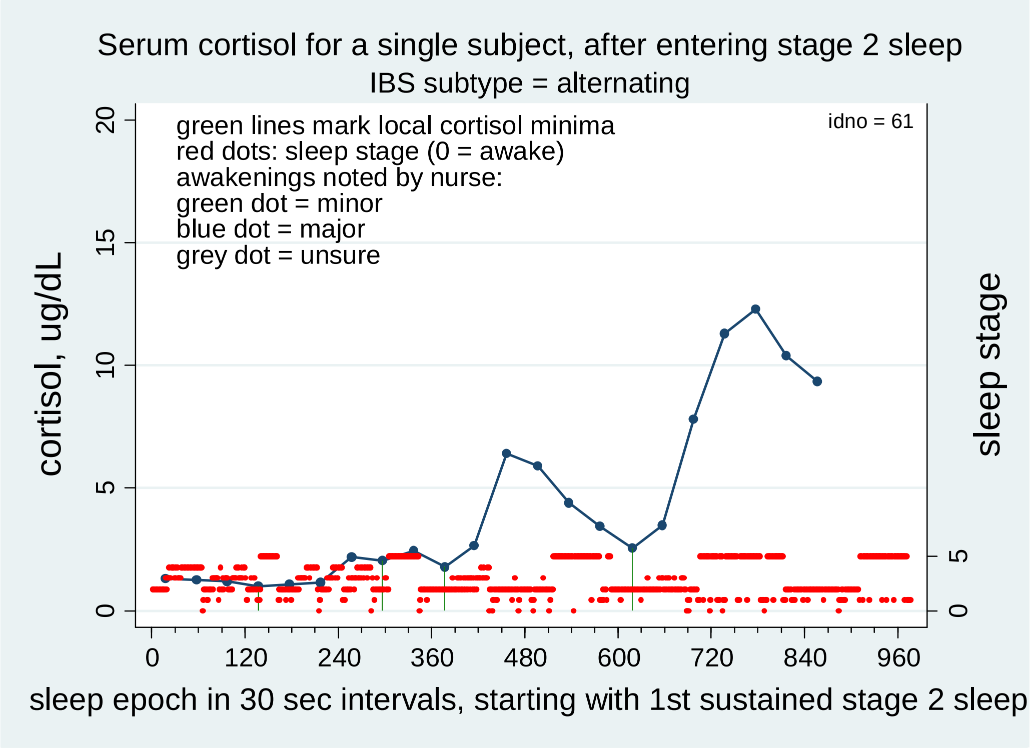

Serum cortisol for a single subject, after entering stage 2 sleep
IBS subtype = alternating
green lines mark local cortisol minima
red dots: sleep stage (0 = awake)
awakenings noted by nurse:
green dot = minor
blue dot = major
grey dot = unsure
idno = 26
cortisol, ug/dL
0
5
10
15
20
sleep stage
0
5
0
120
240
360
480
600
720
840
960
sleep epoch in 30 sec intervals, starting with 1st sustained stage 2 sleep

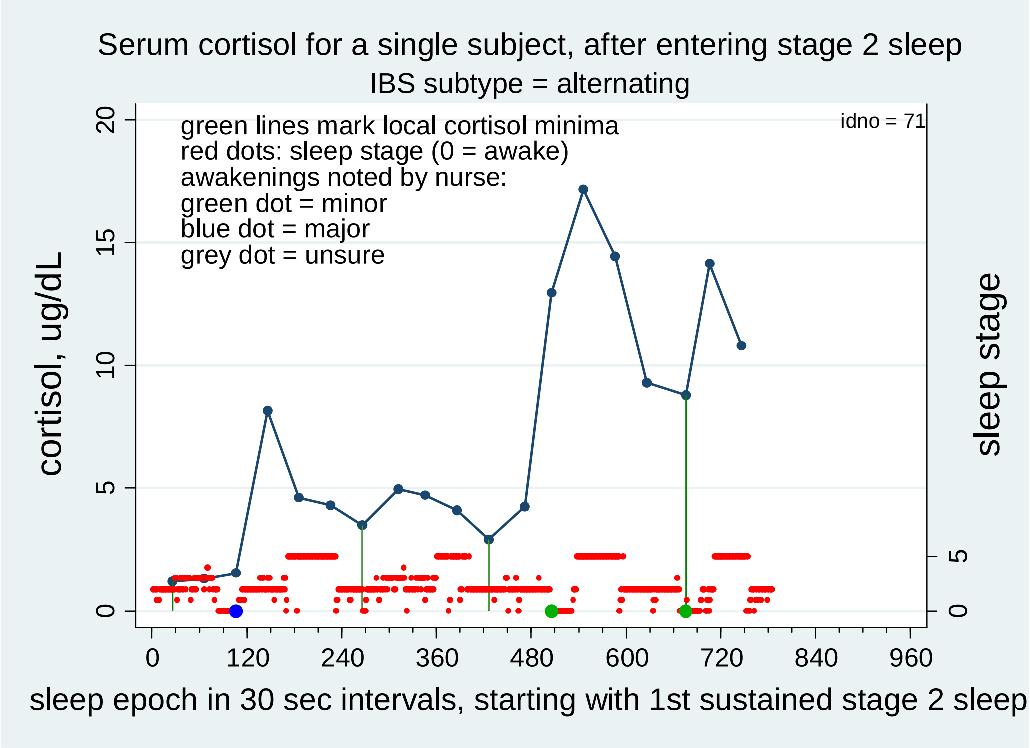

Serum cortisol for a single subject, after entering stage 2 sleep
IBS subtype = alternating
green lines mark local cortisol minima
red dots: sleep stage (0 = awake)
awakenings noted by nurse:
green dot = minor
blue dot = major
grey dot = unsure
idno = 33
cortisol, ug/dL
0
5
10
15
20
sleep stage
0
5
0
120
240
360
480
600
720
840
960
sleep epoch in 30 sec intervals, starting with 1st sustained stage 2 sleep


Serum cortisol for a single subject, after entering stage 2 sleep
IBS subtype = alternating
green lines mark local cortisol minima
red dots: sleep stage (0 = awake)
awakenings noted by nurse:
green dot = minor
blue dot = major
grey dot = unsure
idno = 34
cortisol, ug/dL
0
5
10
15
20
sleep stage
0
5
0
120
240
360
480
600
720
840
960
sleep epoch in 30 sec intervals, starting with 1st sustained stage 2 sleep


Serum cortisol for a single subject, after entering stage 2 sleep
IBS subtype = alternating
green lines mark local cortisol minima
red dots: sleep stage (0 = awake)
awakenings noted by nurse:
green dot = minor
blue dot = major
grey dot = unsure
idno = 49
cortisol, ug/dL
sleep stage
0
5
10
15
20
0
5
0
120
240
360
480
600
720
840
960
sleep epoch in 30 sec intervals, starting with 1st sustained stage 2 sleep


Serum cortisol for a single subject, after entering stage 2 sleep
IBS subtype = alternating
green lines mark local cortisol minima
red dots: sleep stage (0 = awake)
awakenings noted by nurse:
green dot = minor
blue dot = major
grey dot = unsure
idno = 54
cortisol, ug/dL
sleep stage
0
5
10
15
20
0
5
0
120
240
360
480
600
720
840
960
sleep epoch in 30 sec intervals, starting with 1st sustained stage 2 sleep


Serum cortisol for a single subject, after entering stage 2 sleep
IBS subtype = alternating
green lines mark local cortisol minima
red dots: sleep stage (0 = awake)
awakenings noted by nurse:
green dot = minor
blue dot = major
grey dot = unsure
idno = 61
cortisol, ug/dL
0
5
10
15
20
sleep stage
0
5
0
120
240
360
480
600
720
840
960
sleep epoch in 30 sec intervals, starting with 1st sustained stage 2 sleep


Serum cortisol for a single subject, after entering stage 2 sleep
IBS subtype = alternating
green lines mark local cortisol minima
red dots: sleep stage (0 = awake)
awakenings noted by nurse:
green dot = minor
blue dot = major
grey dot = unsure
idno = 71
cortisol, ug/dL
0
5
10
15
20
sleep stage
0
5
0
120
240
360
480
600
720
840
960
sleep epoch in 30 sec intervals, starting with 1st sustained stage 2 sleep