Supplementary Appendix for:

Social bonds do not mediate the relationship between early adversity and adult glucocorticoids in wild baboons

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1. Visualization of dyadic sociality index (DSI) distribution

Figure S1

**Figure S1:** Dyadic sociality index (DSI) scores for all female-female grooming dyads, and social bond strength scores for four randomly chosen female baboons in the same year. The gray dots represent the DSI scores of each available female-female dyad who groomed together in a given social group, during the year in question. The DSI scores for grooming dyads of an individual focal female (e.g., female ACI, shown in the top facet) are shown in either green (the female’s top three grooming partners during the year) or purple (all other grooming partners). The vertical green line is the female’s score for bond strength with females, which is the mean of her DSI scores with her top three female grooming partners. The dashed black line is the population mean of female scores for bond strength with females, aggregated across all female-years included in our data set. The light blue box to the right of the dashed line represents bond strength scores one standard deviation above the mean, while the pink box to the left of it represents bond strength scores one standard deviation below the mean. For example, ACI’s social bond strength score during the year depicted here was nearly one standard deviation higher than the population mean across all years, while HUC’s was approximately one half of a standard deviation lower.
2. Use of functional principal components (FPCA) results in mediation models

A key innovation in our statistical approach is to summarize the high-dimensional trajectories for social bond strength (the mediator) and fGCs (the outcome) by their first few functional principle components (i.e., those that collectively explain at least 90% of the variation in the trajectories), and use these low-dimensional summary scores in our mediation models. Here we illustrate our use of FPCA and extend the three linear equations described in the ‘Modeling Approach’ section of the main text, to incorporate these functional principal components.

We begin by modeling the relationship between the mediator trajectory $M_{ij}$ and the early adversity $A_i$; this corresponds to equation (1) in the main text. We express the trajectory of the mediator as a combination of covariate effects $C_i \beta_m$, random effects $r_{\text{cluster}}^m$ and $r_{\text{hydro}}^m$ (see ‘Control Variables’ in the main text and ‘Measurement of Covariates and Random Effects’ below), an individual smooth process $M_i'(t_{ij})$, and observed errors $\epsilon_{ij}$.

$$M_{ij} = C_i \beta_m + r_{\text{cluster}}^m + r_{\text{hydro}}^m + M_i'(t_{ij}) + \epsilon_{ij}, \epsilon_{ij} \sim N(0, \sigma_m^2) \quad (1)$$

Figure S2 is a visual representation of three of these social bond strength trajectories, for three randomly chosen individuals: EAG (who experienced no early adversities), OCT (who experienced one source of adversity), and GUI (who experienced three sources). The solid lines are their observed social bond strength values, and the dashed lines are the smoothed processes expressed by the above equation.
Figure S2: Social bond strength trajectories for three randomly chosen individuals. Animal EAG experienced no early adversity, animal OCT experienced one source, and animal GUI experienced two or more sources. The solid lines are the residuals of their social bond strength values over time, after controlling for relevant covariates and random effects (described in the main text under “control variables” and below in “measurement of covariates and random effects”). The dashed lines are the smooth underlying process we assume these values are representative of, expressed in equation 1.

To express the smooth process $M'(t_{ij})$ with a lower dimensional representation, we apply functional principal component analysis (FPCA). Specifically, we express the smooth curve as a linear combination of principal components, by exploiting the correlation structure of the smooth curves in the population. First, we decompose the correlation function of the smooth process, namely the correlation between any two different time points $t_1, t_2$,

$$\text{Cov}(M_i'(t_1), M_i'(t_2)) = \sum_{k=1}^{\infty} \lambda_k \psi_k(t_1)\psi_k(t_2), \lambda_1 \geq \lambda_2 \geq \cdots \geq 0$$

Equation (2)
where $\lambda_k$ is the explained variance of the orthogonal normal principal components $\psi_k(t)$. We arrange the principal components in descending order by the amount of variance they explain. The principal components that explain more variance $\lambda_k$ are more important to express the smooth process. Therefore, we use the first $K$ principal components, where $K$ is the number of components necessary to collectively explain at least 90% of the variance $(\sum_{k=1}^{K} \lambda_k / \sum_{k=1}^{\infty} \lambda_k \geq 90\%)$. In the next step, we express the smooth process of each individual’s trajectory as a linear combination of the $K$ principal components,

$$M'_i(t) = \sum_{k=1}^{K} \xi_{ik} \psi_k(t)$$

where $\xi_{ik}$ is the principal score for individual $i$ on the $k$th principal component. The variance of $\xi_{ik}$ equals the explained variance of principal component, $\lambda_k$. With the help of FPCA, we can efficiently express the smooth process and individual trajectory with a few principal components (in our case, $K$ is never greater than 4), without too much information loss. This leads to the following model,

$$M_{ij} = C_{ij} \beta_m + r_{cluster}^m + r_{hydro}^m + \sum_{k=1}^{K} \xi_{ik} \psi_k(t) + \epsilon_{ij}, \epsilon_{ij} \sim N(0, \sigma_m^2)$$

which corresponds to equation (7) in the main text. Furthermore, we assume that the differences in trajectories between the animals that experienced adversity and those who did not is captured by differences in the principal scores, since other relevant variables have been controlled for when generating the trajectories. Therefore, we have the following specification for the principal scores:

$$\xi_{ik} = A_i(\tau_i^k - \tau_0^k) + \tau_0^k + \eta_{ik}, \eta_{ik} \sim N(0, \lambda_k), \lambda_1 \geq \lambda_2 \geq \cdots \lambda_K \geq 0$$
where $\tau_1^k$ denotes the average $k$ principal score for the units in the adversity group while $\tau_0^k$ represents the non-adversity group. We fit equation (4) simultaneously with equation (5).

Hence, instead of directly inspecting the effect of adversity on the trajectories, which can be of very high dimensions, we examine its effect on each level of principal scores $\xi_{i1}, \xi_{i2}, \ldots, \xi_{iK}$.

We can then transform the differences in the effect on the principal scores to differences in the effect on the trajectories. Based on equations (4) and (5), we can calculate the conditional expectation of mediator trajectory $M_{ij}$ at time point $t_{ij}$ as follows,

$$E(M_{ij}|C_{ij}, A_i) = \beta_mC_{ij} + \sum_{k=1}^K (A_i(\tau_1^k - \tau_0^k) + \tau_0^k) \psi_m(t_{ij}),$$

which corresponds to equation (1) in the main text. Next, we express the effect of early adversity on social bond strength (i.e., the effect on the mediator, also corresponding to equation (1) in the main text) using:

$$b_1(t) = \sum_{k=1}^K (\tau_1^k - \tau_0^k) \psi_k(t)$$

The effect on the mediator also has a time index, since we are estimating the effect of adversity on social bond strength trajectories across the lifespan. Integrating $b_1(t)$ over time gives the estimation of parameter $\beta_1$ (the beta coefficient associated with the effect on the mediator) in equation (1) in the main text:

$$\beta_1 = \frac{1}{T} \int_0^T b_1(t) dt$$

For the outcome variable, fGCs, we use a similar model, except we include the effect of the mediator on the outcome. We employ the following model for the outcome with $S$
principal components. $S$ is equivalent to $K$ in equation 3, but the outcome and mediator variables may have different numbers of components that capture 90% of the variation:

$$Y_{ij} = X_{ij}\beta_Y + r_{\text{cluster}}^Y + \eta_{\text{hydro}}^Y + \gamma M_{ij} + \sum_{k=1}^{S} \zeta_{ik} \eta_k(t_{ij}) + \nu_{ij}, \nu_{ij} \sim N(0, \sigma^2_\nu)$$ (9)

where $\gamma$ represents the bond effect of the mediator variable on fGCs, $\eta_k(t)$ is the $k$th principal components for the outcome process, $\zeta_{ik}$ is the corresponding principal component score for individual $i$, and $\nu_{ij}$ is the observation error for the outcome trajectory. Similar to the model for the principal scores of the mediator, we model the effect of early adversity on the principal score $\zeta_{ik}$,

$$\zeta_{ik} = A_i(\theta^k_1 - \theta^k_0) + \theta^k_0 + \kappa_{ik}, \kappa_{ik} \sim N(0, \rho_k), \rho_1 \geq \rho_2 \geq \rho_3 \cdots \rho_S \geq 0$$ (10)

where $\theta^k_1$ denotes the average $k$ principal score for the baboons in the adversity group while $\theta^k_0$ stands for the animals in the non-adversity group, and $\rho_k$ is the explained variance of component $k$. Figure S3 is a visual representation of fGC trajectories for the same three randomly chosen individuals shown in S2, except the solid lines are now the (covariate-adjusted) fGC values, and the dashed lines are the smoothed process for fGCs, generated by the above equation.
Figure S3: Fecal glucocorticoid (fGC) trajectories for the same three randomly chosen individuals whose social bond strength trajectories are depicted in Figure S2. Animal EAG experienced no early adversity, animal OCT experienced one source, and animal GUI experienced two or more sources. The solid lines are the residuals of their fGC values over time, after controlling for relevant covariates and random effects (described in the main text under “control variables” and below in “measurement of covariates and random effects”). The dashed lines are the smooth underlying process we assume these values are representative of.

Combining (9) and (10), we can calculate the conditional expectation of fGC along its trajectory across the lifespan:

$$E(Y_{ij}|X_{ij}, A_i, M_{ij}) = X_{ij} \beta_Y + \gamma M_{ij} + \sum_{k=1}^{S} (A_i(\theta_1^k - \theta_0^k) + \theta_0^k) \eta_k(t_{ij}),$$

(11)

which corresponds to equation (3) in the main text. We then express the mediation effect $b_2(t)$ (from equation (3) in the main text), which is the product of bond effect $\gamma$ and the effect on mediator $b_1(t)$. Its integral corresponds to $\beta_{1Y}$ from linear equation (3) in the main text:
\[ b_2(t) = \gamma b_1(t) = \gamma \sum_{k=1}^{M} (\tau_1^k - \tau_0^k)\psi_k(t) \]  
\text{(12)}

Mediation effect: \( \beta_1 \gamma: \frac{1}{T} \int_0^T b_2(t) dt \)  
\text{(13)}

The direct effect of early adversity on fGCs \( b_3(t) \), which is any effect of adversity that does not occur via social bond strength, is:

\[ b_3(t) = \sum_{k=1}^{S} (\theta_1^k - \theta_0^k)\eta_k(t) \]  
\text{(14)}

Direct effect: \( \beta_2 - \beta_1 \gamma: \frac{1}{T} \int_0^T b_3(t) dt \)  
\text{(15)}

The integral value of \( b_3(t) \) corresponds to \( \beta_2 - \beta_1 \gamma \) (i.e., the direct effect) in the linear equations in the main text. Finally, we express the total effect of early adversity on fGCs \( b_4(t) \), as a sum of the mediation and direct effects:

\[ b_3(t) = \sum_{k=1}^{S} (\theta_1^k - \theta_0^k)\eta_k(t) + \gamma \sum_{k=1}^{M} (\tau_1^k - \tau_0^k)\psi_k(t) \]  
\text{(16)}

Total effect: \( \beta_2: \frac{1}{T} \int_0^T b_4(t) dt \).  
\text{(17)}

The average value of \( b_4(t) \) is the equivalent of \( \beta_2 \) (i.e., the total effect) in the main text. This establishes the framework necessary for mediation analysis, when social bond strength and fGC are sparse and/or irregular trajectories. With the model specification above, the parameters of interest for mediation analysis are:

Effect on mediator: \( \frac{1}{T} \int_0^T \sum_{k=1}^{M} (\tau_1^k - \tau_0^k)\psi_k(t) dt \),  
\text{(18)}

Bond effect: \( \gamma \),  
\text{(19)}

Mediation effect: \( \frac{1}{T} \int_0^T \gamma \sum_{k=1}^{M} (\tau_1^k - \tau_0^k)\psi_k(t) dt \),  
\text{(20)}

Direct effect: \( \frac{1}{T} \int_0^T \sum_{k=1}^{S} (\theta_1^k - \theta_0^k)\eta_k(t) dt \)  
\text{(21)}
Integrating values over time allows us to express the lifetime effect as a single number, which can be interpreted as, on average, across the lifespan, animals that experience a given source of adversity have mediator (or outcome) values that are x amount higher (or lower) than animals who do not. Visual inspection of the size of the effects across the lifespan, when aggregated across animals, indicated the effects were relatively constant. Figure S4 below shows the size of the total effect of early adversity on fGCs (the blue line), as well as the mediation (i.e., mediation) effect of social bond strength on fGCs, across the life-years that our data cover (ages 4-18).

**Figure S4**

![Effect decomposition plot showing the size of the total effect of early adversity on fecal glucocorticoids (blue line, with dashed black 95% CI lines), and the size of the mediating effect of social bond strength (red line, again with dashed black 95% CI lines), across the female life-years our data cover (ages 4-18). The effect sizes of both are relatively constant across time. These effect sizes are from our model that evaluates animals who experienced no adversities, versus those who experienced any one or more, where social bond strength with other females is treated as the mediator variable (Table 2 in main text).]
3. Results of model comparing females who experienced no adversity to those who experienced two or more

Table S1. Effect sizes and 95% confidence intervals (in brackets) for a model that tests the mediation effect of social bonds with females, comparing animals who experienced no sources of early adversity with those who experienced two or more. Entries in bold have 95% CIs that exclude zero.

| Cumulative adversity (0 vs 2+) | Total effect ($\beta_2$) | Mediation effect ($\beta_1 \gamma$) | Direct effect ($\beta_2 - \beta_1 \gamma$) | Effect on mediator ($\beta_1$) | Bond effect ($\gamma$) |
|-------------------------------|--------------------------|-----------------------------------|------------------------------------------|----------------------------|------------------------|
| 0.212 | 0.007 | 0.205 | -0.224 | -0.054 |
| 0.008, 0.415 | [-0.013, 0.022] | 0.018, 0.392 | [-0.437, -0.011] | [-0.118, -0.003] |

The column headings in Table S1 match the color of the relationship arrow they correspond to in Figure 2 in the main text.

4. Check for the assumption of sequential unconfoundedness

We tested for the possibility of feedback between the fGC and social bond processes, a scenario in which the sequential unconfoundedness assumption described in the ‘Causal assumptions’ section of the main text would be violated. Specifically, we added 1) the most recent prior observed fGC value (Table S1), or 2) the average of all past observed fGC values (Table S2), as a predictor in the mediation model (Equation 4 above). Both of these models led to negligible differences in the results from the ones reported in the main text, and thus provide evidence against feedback between the fGC and social bond strength processes.
Table S2. Effect sizes and 95% confidence intervals (in brackets) for models that test the mediation effect of social bonds with females, including the most recent prior observed fGC value. Entries in bold have 95% CIs that exclude zero.

|                      | Total effect (β2) | Mediation effect (β1,γ) | Direct effect (β2 - β1,γ) | Effect on mediator (β1,γ) | Bond effect (γ) |
|----------------------|-------------------|-------------------------|---------------------------|--------------------------|----------------|
| **Drought**          | 0.120             | 0.005                   | 0.110                     | -0.168                   | -0.060         |
|                      | [0.010, 0.230]    | [>0.000, 0.010]         | [0.005, 0.215]            | [-0.320, 0.16]           | [-0.092, -0.033] |
| **Competing sibling**| 0.087             | 0.009                   | 0.081                     | -0.103                   | -0.048         |
|                      | [-0.016, 0.205]   | [0.005, 0.012]          | [-0.017, 0.195]           | [-0.260, 0.039]          | [-0.076, -0.021] |
| **High group density**| 0.116             | 0.008                   | 0.101                     | -0.278                   | -0.060         |
|                      | [-0.026, 0.274]   | [0.001, 0.016]          | [-0.033, 0.251]           | [-0.526, 0.030]          | [-0.088, -0.033] |
| **Maternal death**   | 0.064             | 0.014                   | 0.052                     | -0.218                   | -0.047         |
|                      | [-0.020, 0.149]   | [0.009, 0.017]          | [-0.027, 0.134]           | [-0.418, -0.018]         | [-0.071, -0.020] |
| **Low maternal rank**| 0.136             | 0.010                   | 0.129                     | -0.148                   | -0.049         |
|                      | [0.006, 0.267]    | [0.006, 0.014]          | [0.013, 0.244]            | [-0.266, 0.012]          | [-0.079, -0.019] |
| **Maternal social isolation**| 0.029        | -0.004                   | 0.028                     | -0.046                   | -0.062         |
|                      | [-0.049, 0.105]   | [-0.008, -0.001]        | [-0.047, 0.100]           | [-0.182, 0.104]          | [-0.092, -0.036] |
| **Cumulative adversity (0 vs 1+)**| 0.096       | 0.011                   | 0.088                     | -0.098                   | -0.054         |
|                      | [0.003, 0.189]    | [0.008, 0.013]          | [0.008, 0.169]            | [-0.187, 0.008]          | [-0.080, -0.029] |
| **Cumulative adversity (1 vs 2+)**| 0.137       | 0.005                   | 0.129                     | -0.170                   | -0.057         |
|                      | [0.014, 0.259]    | [-0.003, 0.012]         | [0.012, 0.245]            | [-0.352, -0.007]         | [-0.104, -0.009] |

1The column headings in Table S1 match the color of the relationship arrow they correspond to in Figure 2 in the main text.

Table S3. Effect sizes and 95% confidence intervals (in brackets) for models that test the mediation effect of social bonds with females, including the average of all past observed fGC values. Entries in bold have 95% CIs that exclude zero.

|                      | Total effect (β2) | Mediation effect (β1,γ) | Direct effect (β2 - β1,γ) | Effect on mediator (β1,γ) | Bond effect (γ) |
|----------------------|-------------------|-------------------------|---------------------------|--------------------------|----------------|
| **Drought**          | 0.126             | 0.011                   | 0.116                     | -0.162                   | -0.054         |
|                      | [0.010, 0.241]    | [>0.0002, 0.021]        | [0.010, 0.221]            | [-0.309, -0.016]         | [-0.086, -0.027] |
| **Competing sibling**| 0.084             | 0.006                   | 0.078                     | -0.106                   | -0.051         |
|                      | [-0.019, 0.202]   | [0.002, 0.009]          | [-0.020, 0.192]           | [-0.263, 0.036]          | [-0.079, -0.024] |
| **High group density**| 0.124             | 0.016                   | 0.109                     | -0.270                   | -0.052         |
|                      | [-0.018, 0.281]   | [0.002, 0.030]          | [-0.019, 0.252]           | [-0.514, -0.027]         | [-0.081, -0.026] |
| **Maternal death**   | 0.061             | 0.010                   | 0.049                     | -0.222                   | -0.050         |
|                      | [-0.023, 0.146]   | [0.006, 0.013]          | [-0.031, 0.131]           | [-0.433, -0.010]         | [-0.075, -0.024] |
| **Low maternal rank**| 0.132             | 0.006                   | 0.125                     | -0.152                   | -0.053         |
|                      | [0.007, 0.258]    | [0.002, 0.010]          | [0.008, 0.241]            | [-0.270, 0.008]          | [-0.083, -0.023] |
| **Maternal social isolation**| 0.036       | 0.003                   | 0.034                     | -0.039                   | -0.056         |
|                      | [-0.043, 0.111]   | [-0.001, 0.006]         | [-0.041, 0.107]           | [-0.176, 0.111]          | [-0.085, -0.030] |
| **Cumulative adversity (0 vs 1+)**| 0.091       | 0.006                   | 0.083                     | -0.101                   | -0.059         |
|                      | [0.009, 0.173]    | [0.003, 0.008]          | [0.003, 0.163]            | [-0.200, -0.006]         | [-0.085, -0.034] |
| **Cumulative adversity (1 vs 2+)**| 0.141       | 0.009                   | 0.133                     | -0.175                   | -0.052         |
|                      | [0.014, 0.269]    | [0.001, 0.017]          | [0.004, 0.262]            | [-0.336, -0.014]         | [-0.100, -0.005] |

1The column headings in Table S2 match the color of the relationship arrow they correspond to in Figure 2 in the main text.

2Estimates of zero that include > indicate that the direction of the effect was positive, but that the effect size was small enough that rounding to the nearest thousandth means there are no visible non-zero digits.
5. Measurement of covariates and random effects

All data were collected by the Amboseli Baboon Research Project’s experienced observers who recognize each baboon as an individual based on morphological differences. Below we explain how data on each covariate listed in Tables 4 and 5 (in the main text) were collected.

Covariates

**Group density** is known from near-daily census records of all members of each baboon group. The average group size in this data set was 26.28 individuals (SD= 9.43, range= 5 - 50). We also squared this variable and used it as a separate covariate on fGCs, to account for a known non-linear relationship between group size and fGCs (1).

**Mean number of co-resident adult maternal relatives** is determined from census records and maternal relatedness information gathered via direct observation of mothers and offspring. Collection of life history data, including birth, began in 1971, so maternal relatedness is established across multiple generations. The average number of co-resident maternal kin was 1.44 (SD= 1.27, range= 0 - 6.40). This variable is only included when social bond strength with other females is the mediator variable, since this is only expected to influence females’ bonds with other females.

**Reproductive state** (cycling, pregnant, lactating, or subadult) is known from direct, near-daily observation of females’ sexual swellings, changes in their paracallosal skin color, and menstrual bleeding, along with births and nursing behavior. Samples collected during the first week of lactation—i.e., the day of parturition and the six days that followed it—were categorized as pregnant, because fGC values remain similar to what they are during pregnancy for that week (2). Our data set contained 3,119 samples from cycling females, 4,135 from lactating females, and 2,557 from pregnant females. It also contained 52 samples from females who were still subadults, but who began cycling later in the month in which the fecal sample was collected.

**Percent of the prior year with an infant (<3 months old)** is determined from census records, along with observations of births. In our sample, the mean percent was 9.47% (SD=...
10.39, range= 0 - 85.88). In rare cases the percentage can exceed 25% either because the female in question had less than 365 days of data in the observation-year preceding the collection of the relevant fecal sample, or because she lost a young infant near the beginning of the year, got pregnant shortly thereafter, and gave birth again. This variable is only included when social bond strength with other females is the mediator variable, since exploratory analyses indicated this only impacted females’ bonds with other females.

**Percent of the prior year cycling** is determined via direct, near-daily observation of individual females’ sexual swellings, changes in their paracallosal skin color, and menstrual bleeding. The mean percent of the year that females cycled was 35.19% (SD= 34.54, range= 0 - 100). This variable is only included when social bond strength with males is the mediator variable, since this is only expected to influence females’ bonds with males.

**Season** is a binary variable that indicates whether the sample in question was collected in the dry season (June-October), during which almost no rain falls, or the wet season (November-May). The annual rainfall at Amboseli ranges from 141mm to 757mm (mean=348mm; Amboseli Baboon Research Project data). In our data set, 3,856 samples were collected during the dry season, and 6,007 were collected during the wet season.

**Mean maximum temperature** is calculated from daily readings of a min-max thermometer at the Amboseli Baboon Research Project field camp. In our data set, the mean was 32.80° C (SD =1.71, range = 27.97 - 38.07).

**Delta rainfall** is determined by averaging the rainfall during a given three-month window across all years the project has collected rainfall data, and subtracting this mean from the particular window in question. Rainfall information is gathered via a rain gauge at the Amboseli field camp. In our data set, the mean delta rainfall was -13.06mm (SD=57.66, range= -142.40 - 250.52).

**Proportional rank** is determined based on the outcomes of all observed, decided agonistic interactions between adult female baboons. Observers recorded the identities of individuals participating in an agonistic encounter and the outcome of the encounter. If one animal behaved submissively while the other was either aggressive or remained neutral, the interaction was recorded as decided. Any interactions without a clear outcome (e.g., where
both animals displayed submissive signals) were not counted toward dominance rank calculations. Proportional rank is expressed as the proportion of adult females in her group that a female outranks, where the lowest-ranking female = 0, and the highest-ranking female = 1. In our data set, the average proportional rank females held was 0.52 (SD=0.32, range= 0 – 1).

In addition to the above covariates, our exploratory models included hybrid score, which is a measure of an individual’s degree of admixture between *Papio cynocephalus* and *P. anubis* (3). The inclusion of this variable did not change any of the results, and dramatically decreased our sample size since hybrid scores are unknown for many of the subjects, so it was excluded from the models presented in the main text.

**Random effects**

**Social group** is known from near-daily census records of all members of each baboon group. Though baboons are female philopatric and remain in their natal groups after sexual maturity, they may be members of >1 social group due to naturally occurring fissions. Females in the study lived in 12 different social groups.

**Hydrological years** at Amboseli run from November to October. Our data set contained fecal samples collected during every hydrological year between 1999 (i.e., November 1998-October 1999) and 2017 (mean=519.11 sample/year, SD=276.49, range= 11 – 1,018).

**References**

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