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Article

Stressing Glucose: At the Crossroads of Early Life Adversity, HPA Axis Reactivity and Carbohydrate Metabolism

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Abstract: External stressors strongly increase cardiovascular activity and induce metabolic changes that ensure the availability of glucose and oxygen as part of a co-ordinated stress response. Exposure to stress during early life appears to have an exaggerated long-term effect on this response, leading to an increased risk or cardiometabolic disorders.

Here we demonstrate that acute stress induced glucose release is impacted by the early life environment in rodent maternal deprivation and early-life infection models and this was validated in our EpiPath human early-life adversity cohort. In all three models differences in baseline blood glucose levels after ELA exposure were sex dependent. The human ELA model showed higher levels of basal glucose in females, similar to the mouse infection and rat maternal deprivation models. We anticipated that the stress induced glucose rise would be a GC dependent process. However, the kinetics of stress-induced glucose release, peaking 15-28 minutes before cortisol suggest that it is a GC-independent process. We confirmed this by administering an escalating dose of cortisol to a health human cohort, and the inability of an intravenous GC bolus induce a glucose rise in man confirms that it is a rapid, GC independent, process.

In conclusion, we provide a novel perspective on the mechanisms behind stress related metabolic changes and highlights the importance of collecting early life data as a measure to understand an individual's metabolic status in a better light.

Keywords: glucose; cortisol; corticosterone; stress; early life adversity

1. Introduction

External stressors elicit a physiological response that prepares the body for the fight/flight response, disrupting homeostasis. Glucocorticoids (GC) and catecholamines play an important role in driving this stress response by increasing blood pressure and heart rate along with immune and metabolic changes ensuring availability of glucose and oxygen to meet brain and muscle requirements. As a corollary, prolonged exposure to stress impairs the stress response system and this is reflected as changes in metabolic parameters such as glucose [1].

Both the Hypothalamus-pituitary-adrenal (HPA) stress response axis and an individual's metabolic profile adapt to the immediate environment. The Barker theory states that the quality of the first 1000 days of an individual's life (from conception to age 2) predicts their lifelong metabolic status/phenotype [2, 3], and eventual metabolic dysfunction [4]. Chandan et al (2020) estimated that ELA determines "a significant proportion of the

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cardiometabolic and diabetic disease burden" [5, 6]. Early life adversity (ELA) can be defined as a set of stressful events that an individual is exposed to in these first 1000 days that go on to shape their life-long health trajectory, classically thought to be mediated by changes in the HPA axis. While ELA is all encompassing, it can be broken down into four major sub-categories, 1) early life infection (ELI) and increased antigenic load, 2) nutritional stress, 3) environmental exposure (e.g. pollutants etc..), and 4) psychological and psychosocial stress, although many commonly investigated measures such as low socioeconomic status (SES) cover multiple sub-categories [3]. The exact contribution of these factors is very difficult to dissect in humans. Several studies have reported a higher risk of metabolic diseases such as type-2 diabetes and obesity in ELA exposed individuals [7-10]. Psychological stress effect on glucose metabolism are thought to be mediated by insulin, glucagon, GC, and adrenaline [11, 12]. Fasting-induced gluconeogenesis is orchestrated by the cAMP / PKA / CREB / CRTC2 signalling pathway and is mediated by glucagon. Post-prandial increase of blood insulin levels suppress gluconeogenesis by downregulating gluconeogenic transcription factors such as FOXO1, PGC1α, and CRTC2 [13], and triggering uptake of glucose via GLUT (glucose transporter) receptors [14]. Gluconeogenesis is also triggered during stress along with glycogenolysis, which rapidly subsides [15]. Thus gluconeogenic processes are thought to be the principal mechanism behind the increase in glycaemic measures [15]. However, chronic stress results in consistently high blood glucose levels even up to 6 months after cessation in both rat models of chronic stress [16], and in women where allostatic load positively associates with blood glucose levels [17]. Thus, we have previously suggested that gluconeogenesis may represent a sensitive biological mechanism, which when disturbed can act as a double-edged sword [18]. Recently, Cui et al. showed that synthetic GCs drove the expression of *Klf9* (Kruppel like Factor 9) and *Pgc1α* (PPARG Coactivator 1 Alpha) to promote gluconeogenesis, indicating that gluconeogenesis required gene transcription [13], somewhat at odds with the rapid release of glucose during psychosocial stress [18].

While there are numerous human adversity paradigms, one of the most prevalent is the institutionalisation-adoption model [19-23]. Here, we use the EpiPath cohort of institutionalised-adopted individuals that were investigated some 24 years post adoption. Adopted individuals in the EpiPath cohort had a hyporesponsive HPA axis [24], a clear immune-phenotype reminiscent of accelerated immune ageing [25, 26], although GC signalling and the associated glucocorticoid receptor levels and epigenetic status were unperturbed [27]. These two clear physiological sequelae of ELA can be modelled in vivo. The psychosocial aspect of ELA can be studied in laboratory models of maternal separation and the effects of early life infection can also be modelled in young animals by infecting them with a pathogen of choice such as H1N1. ELA has been known to be a key programmer of the stress response system and can thus potentially dysregulate glucose metabolism and disturb the energy balance, although the exact extent and mechanism behind this is poorly understood.

In this study, using both animal models of early life adversity (maternal separation and infection) as well as in the EpiPath cohort of individuals that were institutionalised at birth and subsequently adopted, we examine the stress-induced rise in plasma glucose under both normal conditions and after exposure to early life adversity. Additionally, using an escalating series of iv cortisol boluses to a healthy human cohort we followed the (lack of) glucose release, highlighting that the rapid glucose release upon exposure to a stressor is independent of GC production or release.

2. Materials and Methods

2.1. Human participants and animals

Healthy human (adversity negative) pharmacokinetic cohort: We used an existing cohort (ESCO) of 40 healthy participants (20 females and 20 males) data from whom have previously been published [28, 29]. Briefly, at study inclusion a physician using a standard medical examination and interview procedure examined participants. Study exclusion

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criteria were i) psychiatric, cardiovascular, sleep or stress-related disorders, ii) any somatic illnesses, iii) smoking, excessive caffeine consumption, glaucoma, pregnancy, or recent (6 months) consumption of illicit drugs. Participants were assigned randomly to one of five sex-balanced groups: placebo (NaCl 0.9%, Braun, Melsungen, Germany) or hydrocortisone: 3 mg, 6 mg, 12 mg and 24 mg of (Hydrocortison 100 mg, Rotexmedia, Trittau, Germany). Two study participants were included per day, starting with the insertion of a flexible intravenous catheter (Vasofix Braunüle, Braun, Melsungen, Germany) in the right arm between 12:00 and 12:45 (mean time 12:54h ± 00:50 h), when physiological cortisol levels are significantly reduced from the morning peak. After a 45 min rest period the catheter was connected to a syringe pump (Infusomat fm, Braun, Melsungen, Germany). Infusion time was approximately 2 min. EDTA anti-coagulated blood samples were drawn through the iv catheter. The study was approved by the Rheinland-Pfalz state Ethical Committee and performed according to the Declaration of Helsinki. All participants provided written informed consent. Participants received a small monetary reward for participation.

Human early life adversity cohort: We used our previously described EpiPath early-life adversity cohort [24-27, 30]. Briefly, EpiPath is a cohort of 115 participants, median age 24 (IQR 20-25.5) years with or without exposure to ELA that was operationalised as "separation from parents in early life and subsequent adoption". The vast majority of ELA subjects had institutionalization or unstable foster care. In this cohort, age of adoption has successfully been used as a proxy for the intensity of ELA experienced [24-27, 30]. The socially evaluated cold pressor test (SECPT) was performed as previously described [24]. Briefly, subjects placed both feet into ice-cooled water (2-3°C) for a period of 3-minutes. Saliva was collected 5 minutes prior to stress onset (-5min), upon stress cessation (+3 min), then at 15, 30, 60, 120 and 180 minutes after stress onset. EpiPath was approved by the Luxembourg National Research Ethics Committee (CNER, No 201303/10 v1.4) as well as the University of Luxembourg Ethics Review Panel (ERP, No 13-002). All participants provided written informed consent in compliance with the Declaration of Helsinki. All study participants received a small financial compensation for their time and inconvenience.

Rats: Pregnant Wistar dams from Janvier Labs (Le Genest-Saint-Isle, France) were housed in plastic cages (Tecniplast, Varese, Italy) with dimensions 48 x 37.5 x 21 centimetres with access to food and water *ad libitum*, maintained under a 12h light-dark cycle (49-54% humidity and 21° C). Animals were left undisturbed for the entire length of the pregnancy except to conduct routine husbandry. At gestational day 16, nesting material was provided, and cages were not further changed until post-natal day (PND) 2. After natural delivery, litters sizes were adjusted to 12/dam and attributed to one of the three treatment groups- control, maternal deprivation for 15 min (MD_{15}) or maternal deprivation for 180 min (MD_{180}). Animals were subjected to a 1-hour restraint stress on PND49 +/- 1 day during the inactive light phase (9 am to 12 pm) and blood was collected from the tail vein in the pre- and post-stress condition. Psychosocial stress modelling in the form of maternal separation was conducted as previously described [31].

Mice: BALB/cJRj mice were originally obtained from Janvier labs (Genest-Saint-Isle, France), and subsequently bred in house. All animals had access to standard rodent chow (#801722; SDS Diets, Witham, UK) and drinking water ad libitum. Pups were then assigned to one of two groups- the test group that was subjected to H1N1 and the controls groups that were administered saline as previously described [32]. On PND42 (±1) a restraint stress test was performed by placing the animals in a transparent tube (according to the size of the animal: Ø2.86cm; BioSeb, Vitrolles, France or Ø2.48cm; G&P Kunsstofftechnik, Kassel, Germany) for 1-hour without the use of anesthesia. All animal experimental procedures were approved by the institutional animal welfare structure and performed in accordance with the European Union directive 2010/63/EU as well as local guidelines.

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2.2. Assessment of cortisol/corticosterone and glucose levels

Glucose levels were measured using a commercial diagnostic electronic glucometer (Accu-Chek, Roche). Human glucose levels were measured from plasma. Briefly, upon thawing, plasma was vortexed and then placed on a fresh Accu-Chek strip. In both rats and mice, glucose and corticosterone measurements were performed after approx. 3 min and 60 min of restraint on blood drawn from the tail vein. A single blood drop from the site of venipuncture was used to measure glucose levels. All blood samples were centrifuged at 2000 x g for 5 minutes and the plasma collected and stored at -80°C, until corticosterone analysis. Plasma corticosterone levels were measured by ELISA (IBL International, Hamburg, Germany), according to the manufacturer's instructions. Human plasma cortisol levels were measured by Cortisol ELISA kit (Enzo, New York, USA) according to the manufacturer's instructions.

2.3. Data presentation and statistical analysis

Data were tested for normality and lognormality using the Kolmogorov Smirnov test. Outliers were identified using the ROUT method (Q = 1-10%) and removed. A paired t-test was conducted to explore blood glucose changes before and after stress, and a 2-way ANOVA was calculated to investigate interactions between different ELA groups and stress induced changes in blood glucose. Additionally, baseline blood glucose differences between ELA and control groups separated by sex were investigated using a Mann-Whitney test/unpaired t-test. Area under the curve was calculated as previously described by Pruessner et al. [33]. All statistical analyses was performed using GraphPad Prism (version 8.2.0 (435)).

3. Results

3.1. Stress results in elevated blood glucose levels

Glucose producing processes are activated under stress conditions to ensure a steady supply of energy to the brain. In mice and rats, this is reflected by a significant increase in blood glucose level after a 1-hour restraint stress (Fig. 1A, $p < 0.0001$; paired t-test and Fig. 1B, $p = 0.0089$; paired t-test). We had previously shown that the socially evaluated cold pressor test used in the EpiPath study increased blood pressure, heart rate and plasma cortisol levels [34]. It also induced a clear trend towards increased blood glucose levels (Fig. 1C). Blood glucose levels rapidly rise after the onset of stress, peaking at 15 minutes with a mean difference of 28 mg/dL (Fig. 1C, $p = 0.0004$, paired t-test compared to t=0), returning to baseline after approximately 3 hours. The SECPT lasted only 3 minutes, explaining the different kinetics from the rodent 1h restraint stress tests. When we extracted the time taken to achieve cortisol peak and glucose peak (Fig. 2A and Fig.2B) and we clearly see that most participants show a cortisol peak 30 minutes after stress onset, while glucose peaks were observed in the 3 or 15 minute post stress samples. Furthermore, this stress induced rise in blood glucose was independent of sex in mice, rats and humans (Fig 3A-F: $p < 0.05$ for all groups (except Fig 3C: female rats, $p = 0.06$; paired t-tests).

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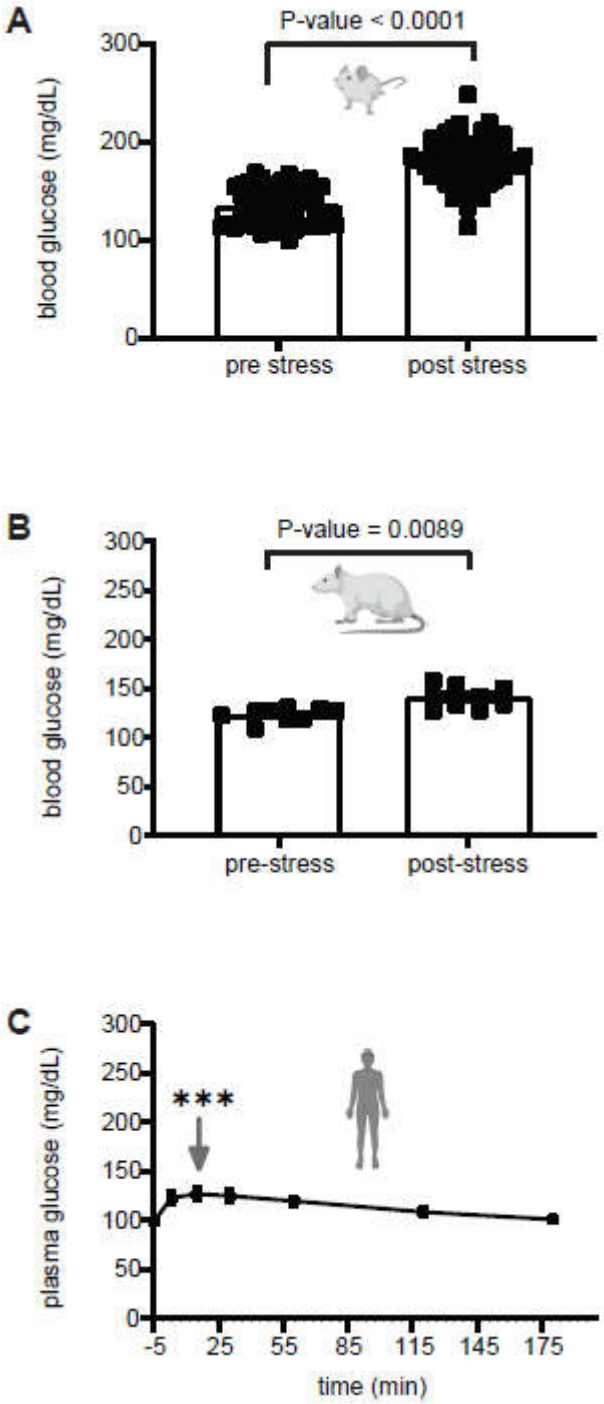

Figure 1. Exposure to stress triggers gluconeogenic processes resulting in increased blood glucose levels. A 1-hour restraint stress in both A) mice and B) rats resulted in elevated blood glucose in the post stress condition. C) A socially evaluated cold pressor test (indicated as a grey box) in humans also showed a similar trend of elevated blood glucose (indicated with a grey arrow) significantly peaking at t = 15 minutes (data not shown, p = 0.0005, paired t-test), after stress that eventually came back to baseline. All data are means along with the standard error of mean (SEM). All measurements have been expressed in mg/dL. Each square represents an individual animal/participant.

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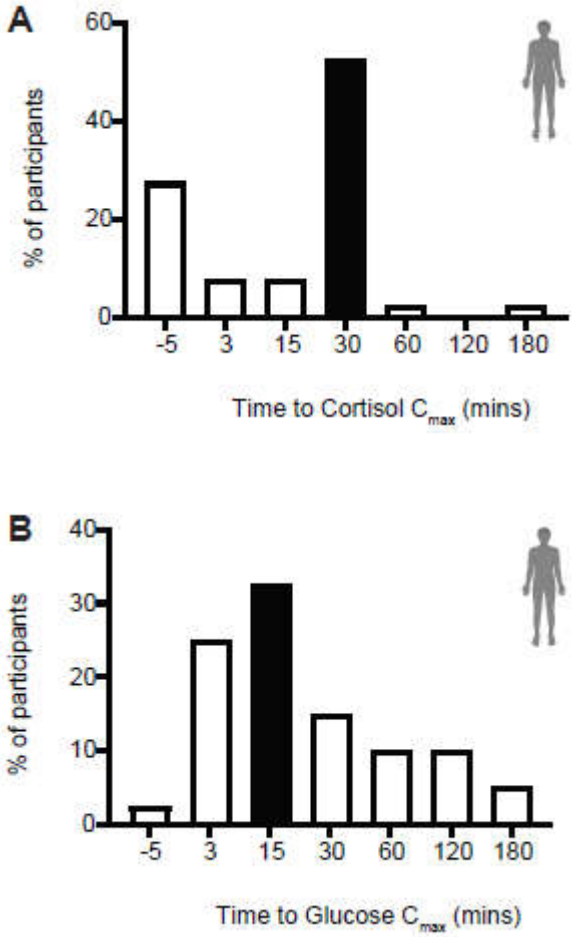

Figure 2. Glucose maximum concentration (Cmax) peak precedes cortisol maximum concentration (Cmax) peak. Bar graph showing % of participants along with the time (minutes) required for them to achieve A) peak cortisol levels and peak plasma glucose levels after the socially evaluated cold pressor test. Black bar represents the time point for which maximum participants achieve peak glucose levels.

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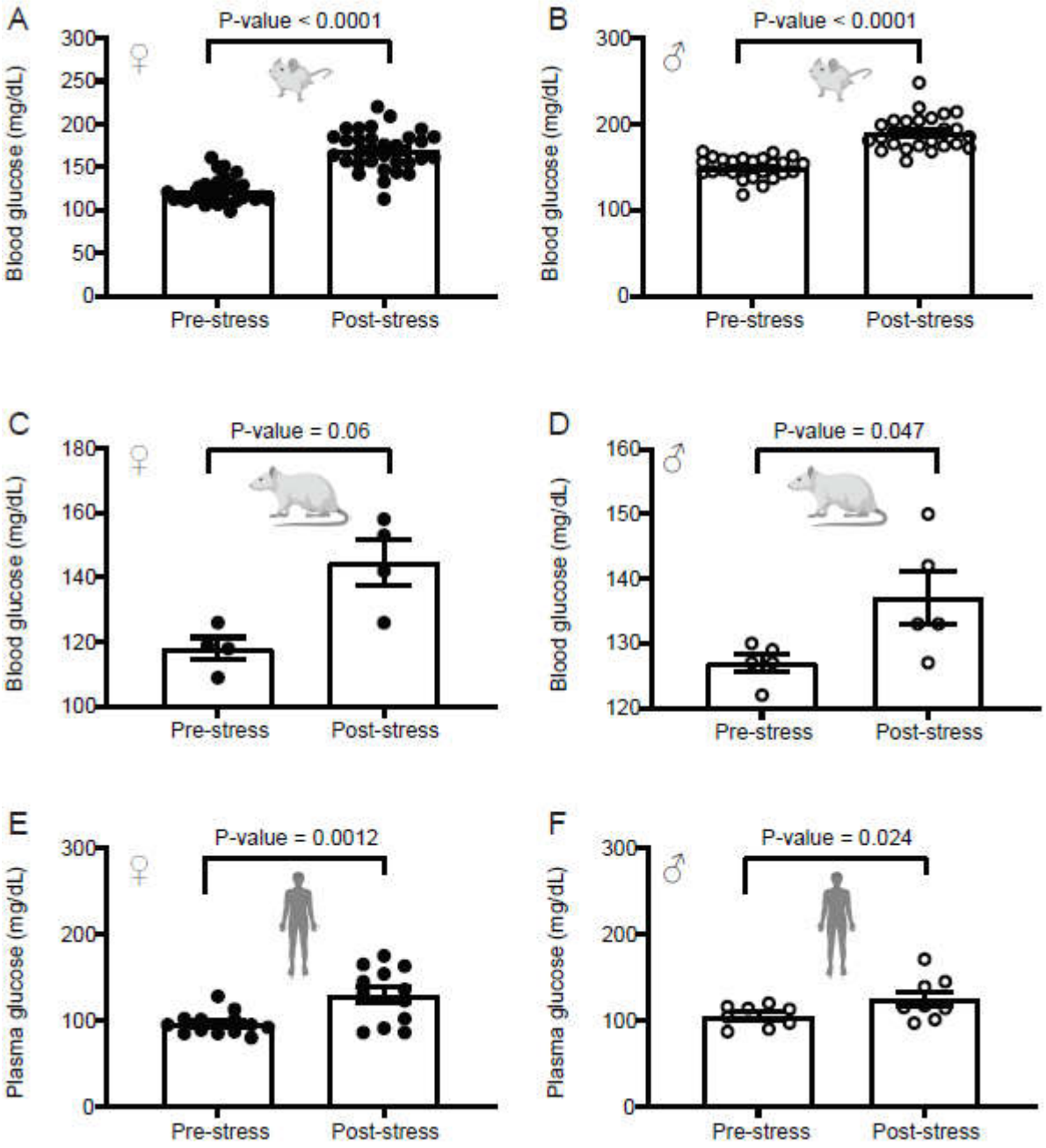

Figure 3. Stress induces glucose release is independent of sex. Blood glucose rise post stress was observed in A) female mice, B) male mice, C) female rats, D) male rats, E) human females and F) human males. All data are means along with the standard error of mean (SEM). All measurements have been expressed in mg/dL. Each dot represents individual animals (females = filled circles, males = empty circles).

3.2. The effect of ELA on baseline blood glucose levels

As ELA is known to influence the functioning of the HPA axis and carbohydrate metabolism, we measured baseline and stress-induced blood glucose for all our adversity models. As reported previously [32], there was a sex difference in baseline glucose levels between control and H1N1-exposed female mice (Fig. 4A: $p = 0.0051$; Mann-Whitney test), with 14% higher baseline glucose levels in H1N1 females. In contrast, the male H1N1-exposed mice showed no difference in baseline blood glucose levels compared to the control males (Fig. 4B: $p > 0.05$; Mann-Whitney test). Furthermore, we found significant differences in baseline blood glucose levels between H1N1 females and males, where the H1N1 males showed approximately 8% higher median basal glucose levels than the

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females (Fig. 4C: p = 0.0056; Mann-Whitney test). This was confirmed in the 2-way ANOVA analysis, with a clear sex effect and group*sex interaction, although without an effect of ELA alone (2-way ANOVA; group effect p = 0.28; sex effect p < 0.0001; group*sex interaction p = 0.0041, data not shown).

Interestingly, although there was no significant MD-induced difference in baseline glucose for female rats (Fig 4D, p>0.05, Kruskal-Wallis test), we saw a significant difference between the male control and MD_{180} rats. Here, MD decreased median baseline glucose levels by 7.5% (Fig. 4E: overall p = 0.015; p for 3h MD group compared to control = 0.013; Kruskal-Wallis test, Dunn's multiple comparison). Similar to the H1N1 male mice (Fig. 4C), MD_{15} male rats showed approximately 9% higher median basal glucose levels than the females (Fig. 4F: p = 0.022; Mann-Whitney test). However we do not see this sex dependent baseline difference in the MD_{180} group (Fig. 4G: p > 0.05; Mann-Whitney test). Interestingly, similar to the mice, the female rats in both MD_{15} and control group showed lower baseline blood glucose levels compared to male rats belonging to both groups (2-way ANOVA; group effect p = 0.095; sex effect p = 0.0042; group*sex interaction p = 0.68, data not shown). Although, our confirmatory 2-way ANOVA analysis did not show a significant group*sex interaction for our our MD_{15} rats, we saw a clear sex effect.

In a manner similar to the H1N1 female mice, female adoptees in the EpiPath cohort had a significantly higher baseline blood glucose level (Fig. 4H: p = 0.0091; Mann-Whitney test), by approximately 12% higher median baseline glucose levels. However, there was no significant difference between the male Epipath adoptees for baseline glucose (Fig. 4I: p > 0.05; Mann-Whitney test). We also saw no sex dependent differences in basal glucose levels for the ELA groups (Fig. 4J: p > 0.05; Mann-Whitney test). It is also interesting to note that previous studies have reported sex differences in blood glucose levels (OGTT) between men and women (reviewed in [35]).

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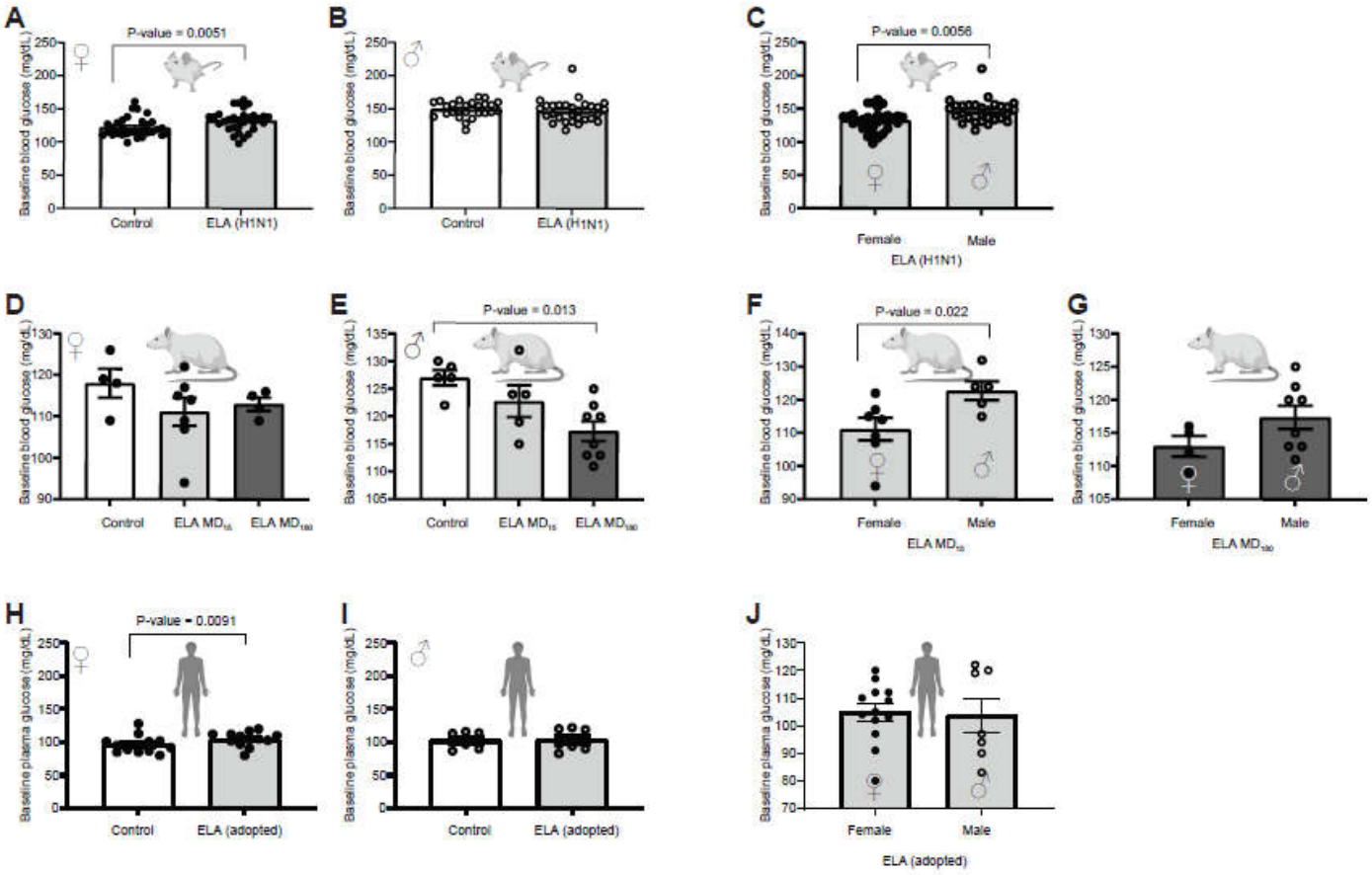

Figure 4. ELA affects baseline blood glucose levels in a sex dependent manner. Baseline blood glucose levels (mg/dL) in A) female H1N1 and control mice, B) male H1N1 and control mice, C) female H1N1 and male H1N1 mice, D) female maternally separated rats (MD_{15} and MD_{180}) and control rats, E) male maternally separated rats (MD_{15} and MD_{180}) and control rats, F) female MD_{15} rats and male MD_{15} rats, G) female MD_{180} rats and male MD_{180} rats, H) female EpiPath participants, I) male EpiPath participants and J) female EpiPath adoptees and male EpiPath adoptees. Each dot represents individual animal/participant. For panels C), F), G) and J), filled circles denote females, while males are represented as empty circles. Data are mean along with the SEM. The different coloured bars indicate the different groups (ELA = grey, controls = white).

3.3. The effect of ELA on the stress-induced rise in blood glucose levels

Mice subjected to an early life H1N1 infection showed a significant rise in blood glucose after a 1-hour restraint stress similar to control mice (Supplementary Fig. S1A: 2-way ANOVA; group effect $p = 0.6$; stress effect $p < 0.0001$; group*stress interaction $p = 0.4$). As baseline glucose levels were sex-dependent, we analysed the stress induced glucose release in for both sexes independently. In female mice, post stress there was a clear H1N1 and stress effect although the interaction term narrowly missed significance (Supplementary Fig. S1B: 2-way ANOVA; group effect $p = 0.28$; stress effect $p < 0.0001$; group*stress interaction $p = 0.06$). In male mice there was a clear H1N1 and stress effect although the interaction term was not significant (Supplementary Fig. S1C: 2-way ANOVA; group effect $p < 0.05$; stress effect $p < 0.0001$; group*stress interaction $p = 0.4$). Overall, there was no significant difference in the delta glucose between the controls and H1N1 mice (Supplementary Fig. S1D: $p > 0.05$; unpaired t-test). Also, baseline blood glucose levels showed no correlation with basal plasma corticosterone (Fig. S1E: $p = 0.42$; $R^2 = 0.018$; linear regression). Neither group showed any definite clustering.

Rats subjected to maternal separation as a psychosocial stress in early life showed a significant rise in blood glucose levels for the control and MD_{180} groups but not for the MD_{15} group Supplementary Fig. S2A: $p = 0.049$ for controls and $p = 0.0077$ for the 3h MD group post stress; 2-way ANOVA; group effect $p = 0.058$; stress effect $p < 0.0001$;

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group*stress interaction p = 0.79, Tukey's multiple comparison test) after the restraint stress [31]. Furthermore, we investigated the stress dependent glucose release in MD animals filtered by sex similar to the mice. In female rats, there was no difference in the stress induced blood glucose rise between the control and any of the ELA MD groups (Supplementary Fig. S2B: $p > 0.05$ for all groups; 2-way ANOVA; group effect $p > 0.05$; stress effect $p = 0.012$; group*stress interaction $p > 0.05$). However in males, we saw significant differences in glucose levels post stress only for the MD 3h group (Supplementary Fig. S2C: $p < 0.0001$ for the 3h MD group; 2-way ANOVA; group effect $p > 0.05$; stress effect $p < 0.0001$; group*stress interaction $p = 0.042$). The overall change in glucose levels (delta) between any of the 3 groups was not significant (Supplementary Fig. S2D: $p > 0.05$; Dunnett's multiple comparison test). Also, there was no correlation between baseline glucose and corticosterone for any of the groups. None of the groups showed any specific pattern of clustering (Supplementary Fig. S2E: $p = 0.11$; $R^2 = 0.18$; linear regression).

In the EpiPath cohort, there was a clear difference between the ELA and control group in their response to the SECPT (Supplementary Fig. S3A: $p < 0.0008$ for the control group; 2-way ANOVA). However, the ELA group did not show a statistically significant rise in blood glucose levels after a cold pressor test, whereas the control group showed a significant rise of plasma glucose at t = 15 mins, although the ELA and stress interaction term was not significant (group effect $p = 0.8$; stress effect $p < 0.0001$; group*stress interaction $p = 0.2$). Also, we investigated the stress dependent glucose release between the controls and the adoptees filtered by sex similar to the mice and rats. In the female participants, we saw a significant increase of stress induced blood glucose levels in both ELA and control groups (Supplementary Fig. S3B: $p = 0.0009$ for the control group and $p = 0.017$ for the ELA adoptees; 2-way ANOVA; group effect $p > 0.05$; stress effect $p < 0.0001$; group*stress interaction $p = 0.4$). However, in the male participants, there was no significant change in blood glucose levels before and after the cold pressor test for either group (Supplementary Fig. S3C: $p > 0.05$ for both groups; 2-way ANOVA; group effect $p > 0.05$; stress effect $p > 0.05$; group*stress interaction $p = 0.28$). Overall delta glucose between the two groups was insignificant (Supplementary Fig. S3D: $p > 0.05$; Mann-Whitney test). Also, there was no association between glucose and cortisol levels, both at baseline (Supplementary Fig. S3E: $p = 0.028$; $R^2 = 0.11$; linear regression) and there was no clear clustering of either control or ELA groups.

3.4. I.v. cortisol administration failed to raise blood glucose levels

All groups that received i.v. cortisol showed a peak in plasma cortisol levels approximately 18 minutes after the infusion indicating a successful administration (Fig. 5A). The plasma cortisol levels for the 3 mg and 6 mg groups came back to baseline after approximately 100 minutes and 160 minutes after iv cortisol administration. The higher dose groups did not return to baseline plasma cortisol levels by the end of the experiment. Participants who were administered a higher dose exhibited higher peak plasma cortisol levels at t = 75 mins, meaning that the observed plasma cortisol peak was proportional to the administered dose (Fig. 5B. $p = 0.0027$, $R^2 = 0.97$; linear regression). The plasma cortisol AUC_g showed a high correlation with the administered cortisol (Fig. 5C. $p = 0.0009$, $R^2 = 0.98$; linear regression). However, contrary to our hypothesis, none of the 5 groups showed an increase in the level of plasma glucose up to 2.5 hours post cortisol administration, irrespective of the dose administered (Fig. 5D, $p > 0.05$ for all time points and all doses against time of injection at t = 57 mins; Wilcoxon test). We also saw no correlation between the calculated plasma glucose AUC_g and either the dose of cortisol administered (Fig. 5E. $p = 0.85$, $R^2 = 0.015$; linear regression) or the plasma cortisol AUC_g (Fig. 5F. $p = 0.92$, $R^2 = 0.0035$; linear regression).

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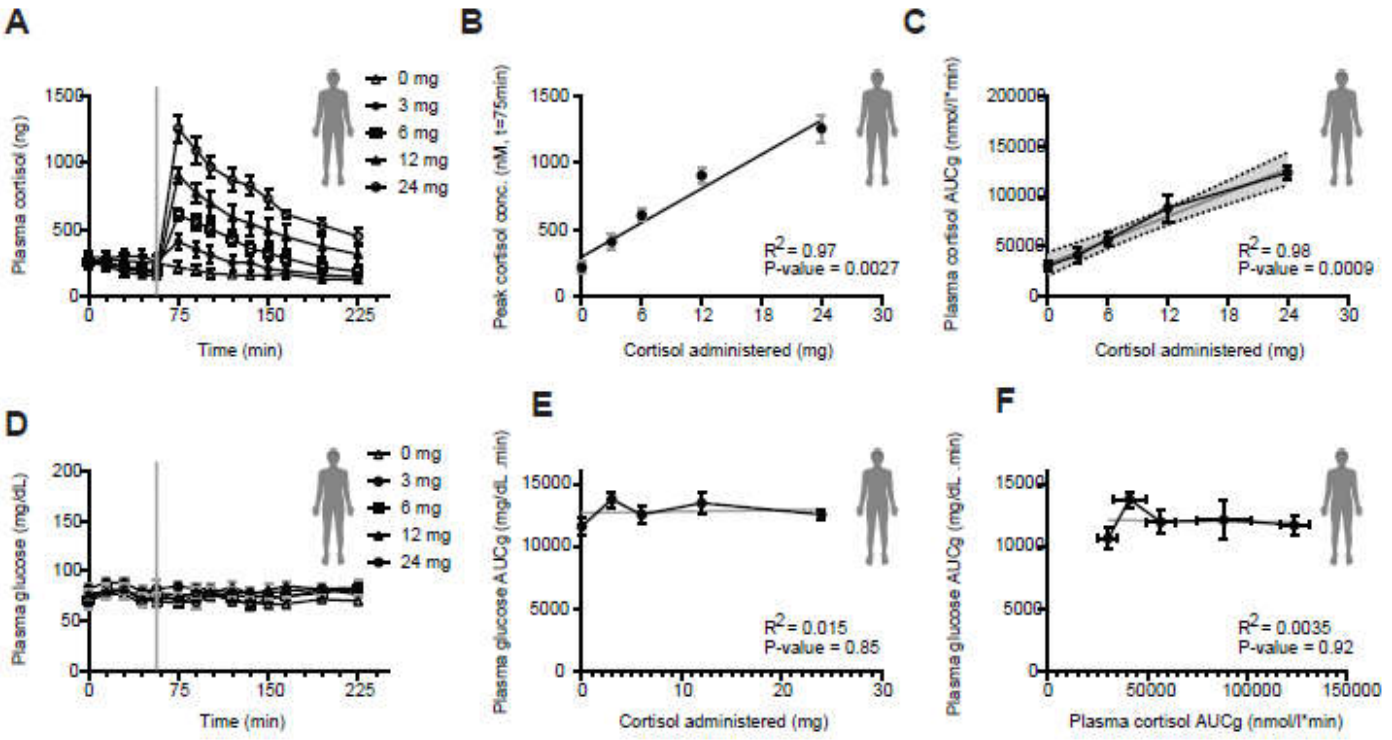

Figure 5. Administered cortisol failed to raise blood glucose levels in (ESCO) participants. A) The plasma cortisol readings (in ng) for the control and the 4 test groups (doses in mg) were reflected correctly in the plasma corresponding to the administered dose (mg). The faint dotted line indicates the time point at which cortisol was infused. The different symbol represents the different doses of administered cortisol. B) The test groups showed a plasma cortisol peak (nmol/l) at approximately 18 minutes after the infusion that was also strongly associated with the administered dose of cortisol (mg). C) There was a clear correlation between calculated plasma cortisol AUC_g (nmol/L.min) and the infused cortisol dose (mg). The grey line represents the line of regression and the light grey area within the dotted lines denotes the area within the error bands. SEM for all panels have been shown as error bars. D) There was no rise in blood glucose level regardless of infused cortisol dose. The faint dotted line indicates the time point (minutes) at which cortisol was infused. The different symbol represents the different doses of administered cortisol. There was no correlation between plasma glucose AUC_g (ng/dL.min) and E) administered cortisol dose (mg) or F) plasma cortisol AUC_g (nmol/L.min). The grey lines for panels B) and C) denote the line of regression. SEM for all panels have been shown as error bars.

3.5. Cortisol administration was functionally active: expression of GR target genes GILZ and FKPB5

As cortisol administration did not induce a glucose rise in the ESCO study participants, we checked the gene expression profile of 2 glucocorticoid receptor target genes known to be induced after GC administration, *GILZ* and *FKPB5*. There was a clear GC-induced gene expression as measured by RT-PCR. For both genes, expression was dose dependent (Fig. 6A. and Fig. 6B), peaking at approximately 195 minutes (135 minutes after injection) although for the highest doses, *FKPB5* expression was still increasing (Fig. 6B). *FKPB5* expression was higher than that of *GILZ* for the same dose of cortisol, however the relative rise was slower than that for GILZ. However, both were significantly dependent on the cortisol dose administered (*GILZ*: Fig. 6C. $p = 0.0011$, $R^2 = 0.98$; linear regression *FKPB5*: Fig. 6D. $p = 0.0014$, $R^2 = 0.98$; linear regression). Also, *FKPB5* expression exhibited a higher percentage of responders to a lower dose of cortisol compared to *GILZ* expression (Fig. 6E. and Fig. 6F).

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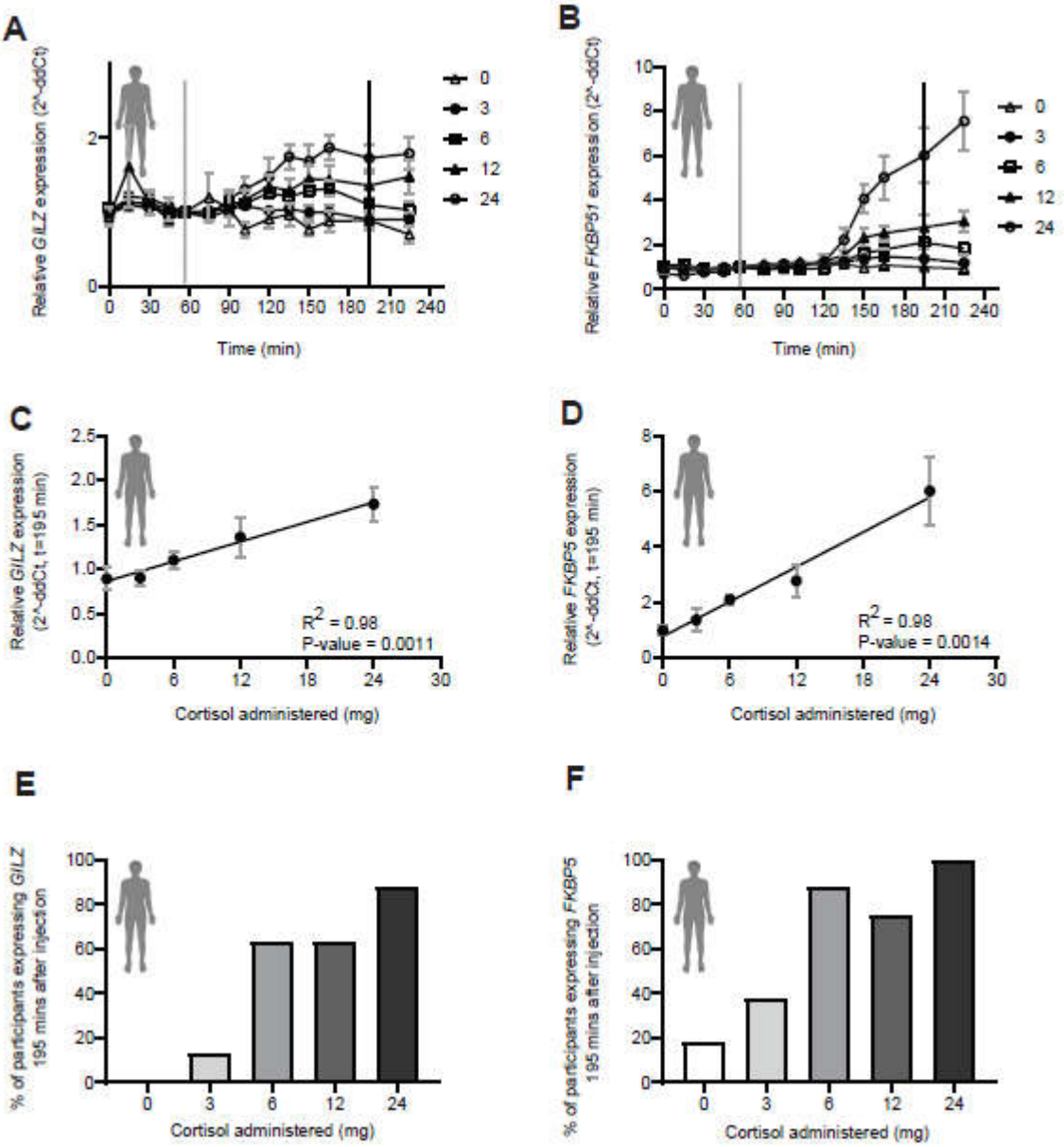

Figure 6. Administered cortisol is functionally active and successful in inducing a GC/GR dependent transcriptional response GR target genes: A) *GILZ* (relative expression) and B) *FKPB5* (relative expression). For both panels, the faint dotted line indicates time point (minutes) at which cortisol was infused. The solid line denotes the best peak of gene expression (195 minutes) and the different symbol represent different doses of administered cortisol. Panels C) and D) show the correlations between the doses of infused cortisol (mg) and C) relative expression of *GILZ* and B) *FKPB5* at 195 minutes. SEM for panels (A-D) have been shown as error bars. Panels E) and F) show the % of responding participants to the infused dose of cortisol (mg), who show gene expression at 195 minutes for E) *GILZ* (relative expression) and F) *FKPB5*. The different colours of the bars indicate the different doses of administered cortisol.

4. Discussion

In this study we demonstrated that acute stress induces a rapid glucose release that could be impacted by the early life environment. Using the MD/ELI models we were able to show baseline and acute stress associated changes in blood glucose that were also validated in our institutionalisation/adoption EpiPath cohort. We also saw significant differences in the baseline blood glucose levels in ELA exposed and control individuals in a sex dependent manner. The early life institutionalisation/adoption model showed higher levels of basal glucose in ELA females, similar to the infection rodent model, while the MD

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males showed a decrease in baseline glucose levels compared to the controls. We anticipated that the stress induced glucose rise would be a GC dependent process, however, the kinetics of glucose release and the inability of an i.v. bolus of GC to induce a glucose rise in man suggests that it is a rapid, GC independent, process.

We recently highlighted a discrepancy in our knowledge. Although there are (limited) data available on how energy availability modulates the stress response [18], knowledge as to how stressors affect energy homeostasis was noticeably absent. It is, however, accepted that the fight/flight response to a stressor requires a rapid mobilisation of resources, mediated through the release of adrenaline and cortisol/corticosterone [18]. At the same time, GC regulates glucose homeostasis, reducing the uptake of glucose by white adipose tissue as well as promoting gluconeogenesis, maintaining glucose availability for central processes [36]. Our data clearly shows this anticipated rapid rise in blood glucose levels after stress. Interestingly, in the EpiPath cohort, this glucose increase slightly preceded peak cortisol levels, while peak cortisol levels were observed 30 minutes after stress onset (Fig. 2A and Fig. 2B), as expected [24, 37], while most participants reached peak glucose levels either 3 or 15 minutes after the onset of stress. This suggests that cortisol does not mediate or initiate glucose release and may not play such an important role in maintaining glucose homeostasis with stress. This was confirmed by our i.v. administration of GC doses up to 24mg. Here, the doses chosen were such that participants receiving 12 or 24 mg cortisol had cortisol levels consistent with a severe psychosocial stressor or excessive physical exercise, while remaining within a range that was physiologically plausible [29, 38]. In the absence of any psychosocial stress signals, the GC bolus did not induce a glucose release. However, the GC bolus was capable of initiating a GC/GR dependent transcriptional response and was therefore functionally active, confirming the theory that cortisol does not mediate or initiate glucose release as the glucose peak precedes cortisol peak in our experimental paradigm. The kinetics of glucose release, with the most rapidly responding participants reaching peak glucose only after 3 minutes, suggests that this is a direct innervation rather than a hormone-mediated process.

Exposure to stress results in long term metabolic and HPA axis changes, and there is growing evidence that the effects are exaggerated when the exposure period coincides with delicate developmental phases, such as in the case of ELA. These changes, reflect the programming or adapting of physiological systems within the body to cope with the environment in which they are born, and represent the basic principal of DOHaD, or the Barker Theory [2, 39]. DOHaD has now been refined into a 3-hit model [3, 40], with the discordance between the environment to which the individual's body has developmentally adapted to and the actual environment later in life being the tipping point between health and disease [41]. We hypothesised that glucose and cortisol are coupled, and that ELA-induced disturbances of the HPA axis would thus alter glucose homeostasis and vice versa. This was a particularly appealing hypothesis as exposure to adversity in the early-life period not only affects the HPA axis, but concurrently induces a long-term metabolic phenotype, and eventual metabolic dysfunction [4]. Furthermore, Chandan et al (2020) estimated that ELA determines "a significant proportion of the cardiometabolic and diabetic disease burden" [5, 6]. In our MD and ELI models, blood glucose was clearly affected by ELA, however, there was no clear unique pattern. Although baseline and stress-induced glucose release were clearly affected by ELA, there was no association with the HPA axis corticosterone response. This suggests that the HPA axis and metabolic stress responses are independently programmed by the early life environment and that the stress-induced glucose response was not coupled to the GC response. Moreover this also implies that the rapid glucose rise to stress is not hormone or signalling mediated. Although a completely different experimental paradigm to those used here would be necessary to investigate this, it is interesting to hypothesise that hepatic innervation [42], with counteracting and counterbalancing sympathetic (splanchnic nerves) and parasympathetic (vagus nerve) inputs may be the explanation behind this. Older data from mice suggests that exposure to an acute psychological stress induces an acute insulin resistance in the liver and not in either muscles or adipose tissues [43]. The most likely reason that this

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is restricted to the liver is that there is an established vagal control of hepatic metabolic processes [44-47]. Transcutaneous stimulation of the auricular vagus nerve was also shown to be able to significantly alter 2-hour plasma glucose, fasting plasma glucose, and glycosylated hemoglobin levels over a 12-week trial period and improve glucose tolerance [48]. Activation of neurons within the dorsal motor nucleus and solitary tract of the vagus have also been reported to actively decrease hepatic gluconeogenesis, as well as modulate the expression of gluconeogenic enzymes, and glucose production [44]. Cardin et. al reported that vagal blockade decreases glucose production in the liver by diminishing glycogenolysis, while gluconeogenic processes remain unaltered [49]. However, a vagal blockade would most probably block afferent fibers, resulting in a decreased sympathetic input to the liver at the same time [49]. This would decrease the stress-associated signal counterbalancing the vagal parasympathetic input, and potentially controlling gluconeogenic processes.

However, any future investigation of neuronal control of hepatic carbohydrate metabolism should also address neuronal glucose sensors such as Glucokinase and GLUT in the hepatoportal region that also regulate glucose metabolism [42], and may have a direct role in modulating the stress induced glucose release. Additionally, glucagon has been shown to mediate (physical) stress induced hyperglycaemia, but can also be hypothesised to orchestrate psychosocial stress induced blood glucose rise [50].

The differences between our EpiPath and ESCO cohorts are striking. As the experimental paradigm used to administer the GC bolus in the ESCO cohort was designed to blind the participant to the pharmacological manipulation being performed, and to induce a strict minimum of psychological stress, the absence of such visual and psychological stimuli are most probably responsible for the absence of the glucose response. Inversely, our EpiPath cohort and both rat and mice models had a clear psychological stress component, hence the glucose response. Thus the stress-induced glucose release can be attributed to the psychosocial element of the stressor activating higher functions.

Unfortunately, once our hypothesis was proven to be incorrect, despite having adequate statistical power, investigation of the potential role of the vagal-splanchnic/Glucokinase/glucagon interaction required a completely different experimental setup that was not available to us. Furthermore, we were limited by the lack of data on vagal tone and splanchnic nerve activity from the EpiPath cohort. Importantly, our results clearly disprove cortisol as the key diabetogenic hormone in stress situations and instead suggests rapid inputs from the nervous system as the key gluconeogenic driver. Additionally, in our study we were able to show using cross species models that exposure to adversity in the early-life period plays an important role in determining baseline glucose levels, with a clear effect of sex. Moreover, we saw the same effect in the human institutionalisation model as the constituent early life infection and early life psychosocial stressor, maternal deprivation. This was most probably due to an ELA induced epigenetic alteration of the glucose sensors, which in turn impacts the direct neuronal input into the liver. Thus in conclusion, our study provides a fresh perspective on the mechanisms behind stress related metabolic changes and highlights the importance of collecting early life data as a measure to understand an individual's metabolic status in a better light.

Supplementary Materials: Figure S1: Effect of early life infection on stress induced glucose release; Figure S2: Effect of early life MD on stress induced glucose release; Figure S3: Effect of early life institutionalisation/adoption on stress induced glucose release.

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References

1. Sandi, C. and J. Haller, *Stress and the social brain: behavioural effects and neurobiological mechanisms.* Nat Rev Neurosci, 2015. **16**(5): p. 290-304.
2. Barker, D.J. and C. Osmond, *Infant mortality, childhood nutrition, and ischaemic heart disease in England and Wales.* Lancet, 1986. **1**(8489): p. 1077-81.
3. Grova, N., et al., *Epigenetic and Neurological Impairments Associated with Early Life Exposure to Persistent Organic Pollutants.* Int J Genomics, 2019. **2019**: p. 2085496.
4. Delpierre, C., et al., *The early life nutritional environment and early life stress as potential pathways towards the metabolic syndrome in mid-life? A lifecourse analysis using the 1958 British Birth cohort.* BMC Public Health, 2016. **16**(1): p. 815.
5. Chandan, J.S., et al., *Increased Cardiometabolic and Mortality Risk Following Childhood Maltreatment in the United Kingdom.* J Am Heart Assoc, 2020. **9**(10): p. e015855.
6. Holuka, C., et al., *The COVID-19 Pandemic: Does Our Early Life Environment, Life Trajectory and Socioeconomic Status Determine Disease Susceptibility and Severity?* Int J Mol Sci, 2020. **21**(14).
7. Alastalo, H., et al., *Cardiovascular health of Finnish war evacuees 60 years later.* Ann Med, 2009. **41**(1): p. 66-72.
8. Eriksson, M., K. Raikkonen, and J.G. Eriksson, *Early life stress and later health outcomes--findings from the Helsinki Birth Cohort Study.* Am J Hum Biol, 2014. **26**(2): p. 111-6.
9. Gardner, R., et al., *Adverse childhood experiences are associated with an increased risk of obesity in early adolescence: a population-based prospective cohort study.* Pediatr Res, 2019. **86**(4): p. 522-528.
10. Deschenes, S.S., et al., *Adverse Childhood Experiences and the Risk of Diabetes: Examining the Roles of Depressive Symptoms and Cardiometabolic Dysregulations in the Whitehall II Cohort Study.* Diabetes Care, 2018. **41**(10): p. 2120-2126.
11. de Guia, R.M., A.J. Rose, and S. Herzig, *Glucocorticoid hormones and energy homeostasis.* Horm Mol Biol Clin Investig, 2014. **19**(2): p. 117-28.
12. Herzig, S., *Liver: a target of late diabetic complications.* Exp Clin Endocrinol Diabetes, 2012. **120**(4): p. 202-4.
13. Cui, A., et al., *Dexamethasone-induced Kruppel-like factor 9 expression promotes hepatic gluconeogenesis and hyperglycemia.* J Clin Invest, 2019. **129**(6): p. 2266-2278.
14. Thorens, B. and M. Mueckler, *Glucose transporters in the 21st Century.* Am J Physiol Endocrinol Metab, 2010. **298**(2): p. E141-5.
15. Sherwin, R.S. and L. Sacca, *Effect of epinephrine on glucose metabolism in humans: contribution of the liver.* Am J Physiol, 1984. **247**(2 Pt 1): p. E157-65.
16. Nirupama, R., M. Devaki, and H.N. Yajurvedi, *Chronic stress and carbohydrate metabolism: persistent changes and slow return to normalcy in male albino rats.* Stress, 2012. **15**(3): p. 262-71.
17. Lopez-Cepero, A., et al., *Changes in Glycemic Load Are Positively Associated with Small Changes in Primary Stress Markers of Allostatic Load in Puerto Rican Women.* J Nutr, 2020. **150**(3): p. 554-559.
18. Seal, S.V. and J.D. Turner, *The 'Jekyll and Hyde' of Gluconeogenesis: Early Life Adversity, Later Life Stress, and Metabolic Disturbances.* Int J Mol Sci, 2021. **22**(7).
19. Reid, B.M., et al., *Persistent skewing of the T-cell profile in adolescents adopted internationally from institutional care.* Brain Behav Immun, 2019. **77**: p. 168-177.
20. Gunnar, M.R., *A commentary on deprivation-specific psychological patterns: effects of institutional deprivation.* Monogr Soc Res Child Dev, 2010. **75**(1): p. 232-47.
21. Koss, K.J., et al., *Social deprivation and the HPA axis in early development.* Psychoneuroendocrinology, 2014. **50**: p. 1-13.
22. Kroupina, M.G., et al., *Adoption as an intervention for institutionally reared children: HPA functioning and developmental status.* Infant Behav Dev, 2012. **35**(4): p. 829-37.
23. van, I.M.H., et al., *Children in Institutional Care: Delayed Development and Resilience.* Monogr Soc Res Child Dev, 2011. **76**(4): p. 8-30.
24. Hengesch, X., et al., *Blunted endocrine response to a combined physical-cognitive stressor in adults with early life adversity.* Child Abuse Negl, 2018. **85**(1873-7757 (Electronic)): p. 137-144.
25. Elwenspoek, M.M.C., et al., *Proinflammatory T Cell Status Associated with Early Life Adversity.* J Immunol, 2017. **199**(12): p. 4046-4055.
26. Elwenspoek, M.M.C., et al., *T Cell Immunosenescence after Early Life Adversity: Association with Cytomegalovirus Infection.* Front Immunol, 2017. **8**(1263): p. 1263.

27. Elwenspoek, M.M.C., et al., *Glucocorticoid receptor signaling in leukocytes after early life adversity.* Dev Psychopathol, 2020. **32**(3): p. 853-863.
28. Yurtsever, T., et al., *The acute and temporary modulation of PERIOD genes by hydrocortisone in healthy subjects.* Chronobiol Int, 2016. **33**(9): p. 1222-1234.
29. Schilling, T.M., et al., *For whom the bell (curve) tolls: cortisol rapidly affects memory retrieval by an inverted U-shaped dose-response relationship.* Psychoneuroendocrinology, 2013. **38**(9): p. 1565-72.
30. Charalambous, E.G., et al., *Early-Life Adversity Leaves Its Imprint on the Oral Microbiome for More Than 20 Years and Is Associated with Long-Term Immune Changes.* Int J Mol Sci, 2021. **22**(23).
31. Fernandes, S.B., et al., *Unbiased Screening Identifies Functional Differences in NK Cells After Early Life Psychosocial Stress.* Front Immunol, 2021. **12**: p. 674532.
32. Merz, M.P., *'A TALE OF ADVERSITY: THE IMPACT OF EARLY LIFE INFECTION BEYOND THE IMMUNE SYSTEM'*. 2022, University of Luxembourg: Esch-sur-Alzette, Luxembourg. p. 146.
33. Pruessner, J.C., et al., *Two formulas for computation of the area under the curve represent measures of total hormone concentration versus time-dependent change.* Psychoneuroendocrinology, 2003. **28**(7): p. 916-31.
34. Hengesch, X., et al., *Blunted endocrine response to a combined physical-cognitive stressor in adults with early life adversity.* Child Abuse Negl, 2018. **85**: p. 137-144.
35. Mauvais-Jarvis, F., *Gender differences in glucose homeostasis and diabetes.* Physiol Behav, 2018. **187**: p. 20-23.
36. Kuo, T., et al., *Regulation of Glucose Homeostasis by Glucocorticoids.* Adv Exp Med Biol, 2015. **872**: p. 99-126.
37. Kirschbaum, C., K.M. Pirke, and D.H. Hellhammer, *The 'Trier Social Stress Test'--a tool for investigating psychobiological stress responses in a laboratory setting.* Neuropsychobiology, 1993. **28**(1-2): p. 76-81.
38. Kirschbaum, C. and D.H. Hellhammer, *Salivary cortisol in psychoneuroendocrine research: recent developments and applications.* Psychoneuroendocrinology, 1994. **19**(4): p. 313-33.
39. Barker, D.J.P., *Editorial: The Developmental Origins of Adult Disease.* European Journal of Epidemiology, 2003. **18**(8): p. 733-736.
40. Daskalakis, N.P., et al., *The three-hit concept of vulnerability and resilience: toward understanding adaptation to early-life adversity outcome.* Psychoneuroendocrinology, 2013. **38**(9): p. 1858-73.
41. Hochberg, Z., et al., *Child health, developmental plasticity, and epigenetic programming.* Endocr Rev, 2011. **32**(2): p. 159-224.
42. Yi, C.X., et al., *The role of the autonomic nervous liver innervation in the control of energy metabolism.* Biochim Biophys Acta, 2010. **1802**(4): p. 416-31.
43. Li, L., et al., *Acute psychological stress results in the rapid development of insulin resistance.* J Endocrinol, 2013. **217**(2): p. 175-84.
44. Pocai, A., et al., *A brain-liver circuit regulates glucose homeostasis.* Cell Metab, 2005. **1**(1): p. 53-61.
45. Matsuhisa, M., et al., *Important role of the hepatic vagus nerve in glucose uptake and production by the liver.* Metabolism, 2000. **49**(1): p. 11-6.
46. Li, J.H., et al., *Hepatic muscarinic acetylcholine receptors are not critically involved in maintaining glucose homeostasis in mice.* Diabetes, 2009. **58**(12): p. 2776-87.
47. Li, X., et al., *Intracerebroventricular leptin infusion improves glucose homeostasis in lean type 2 diabetic MKR mice via hepatic vagal and non-vagal mechanisms.* PLoS One, 2011. **6**(2): p. e17058.
48. Huang, F., et al., *Effect of transcutaneous auricular vagus nerve stimulation on impaired glucose tolerance: a pilot randomized study.* BMC Complement Altern Med, 2014. **14**: p. 203.
49. Cardin, S., et al., *Involvement of the vagus nerves in the regulation of basal hepatic glucose production in conscious dogs.* Am J Physiol Endocrinol Metab, 2002. **283**(5): p. E958-64.
50. Harp, J.B., G.D. Yancopoulos, and J. Gromada, *Glucagon orchestrates stress-induced hyperglycaemia.* Diabetes Obes Metab, 2016. **18**(7): p. 648-53.