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Effects of a poverty-alleviation intervention on salivary cortisol in very low-income children

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Abstract

Correlational studies have shown associations between social class and salivary cortisol suggestive of a causal link between childhood poverty and activity of the stress-sensitive hypothalamic-pituitary-adrenocortical (HPA) system. Using a quasi-experimental design, we evaluated the association between a family's participation in a large-scale, conditional cash transfer program in Mexico (*Oportunidades*, formerly Progresa) during the child's early years of life and children's salivary cortisol (baseline and responsivity). We also examined whether maternal depressive symptoms moderated the impact of program participation. Low-income households (income <20th percentile nationally) from rural Mexico were enrolled in a large-scale poverty-alleviation program between 1998 and 1999. A comparison group of households from demographically similar communities was recruited in 2003. Following 3.5 years of the *Oportunidades* program, three saliva samples were obtained from children age 2-6 years old from intervention and comparison households (n=1197). Maternal depressive symptoms were obtained using the Center for Epidemiologic Studies-Depression Scale (CES-D). Children who had been in the *Oportunidades* program had lower salivary cortisol levels when compared with those who had not participated in the program, while controlling for a wide range of individual-, household- and community-level variables. Reactivity patterns did not differ between intervention and comparison children. Maternal depression moderated the association between *Oportunidades* program participation and baseline salivary cortisol in children. Specifically, there was a large and significant *Oportunidades* program effect of lowering cortisol in children of mothers with high depressive symptoms but not in children of mothers with low depressive symptomatology. These findings provide the strongest evidence to date that the economic circumstances of the family impact the child's developing stress system and provide a mechanism through which poverty early in life could impact life-course risk for physical and mental health disorders.

Keywords

Mexico; low social status; poverty alleviation intervention; conditional cash transfer program; salivary cortisol; children

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Introduction

Poverty and Child Outcomes

The effects of growing up in poverty extend from infancy through adolescence and into adulthood (Bradley & Corwyn, 2002) and include poor health, cognitive and socio-emotional delays and behavioral problems (Evans, 2004). For most child health outcomes, there is a gradient in which lower family socio-economic status (SES) and material well-being is associated with concurrently poorer outcomes (Chen & Matthews, 2006). Experiencing poverty during childhood also appears to have an independent effect on later health outcomes including decreased life span and increased morbidity, even while controlling for adult SES or social status (Poulton, Caspi, Milne, Thomson, Taylor, Sears et al., 2002). Children growing up in poverty, whether indexed by the family's income or, in low technology cultures, by other indices of the family's material well-being are exposed to a wide range of risk factors, and these conditions are exacerbated in the developing world (Walker, Wachs, Gardner, Lozoff, Wasserman, Pollitt et al., 2007). An estimated 219 million children worldwide (39% of all children in developing countries) have evidence of signs of poor development at multiple levels (Grantham-McGregor, Cheung, Cueto, Glewwe, Richter, Strupp et al., 2007).

Salivary Cortisol as a Marker of Stress System Activity

The multiple mechanisms through which poverty produces poor outcomes are being actively explored. Lack of income leading to deficiencies in material well-being (e.g., inadequate housing, medical care, food, clothing) is associated with greater exposure to stressors (Elder, 1985). These stressors, ranging from increased exposure to pathogens to increased exposure to violence, activate the hypothalamic-pituitary-adrenocortical (HPA) system, which is one of the primary components of the mammalian stress response system (Gunnar & Davis, 2003), and is one mechanistic pathway that has received significant theoretical attention (McEwen & Seeman, 1999). When the brain first perceives a stressor, neural input to the hypothalamus induces secretion of corticotropin releasing hormone (CRH), which stimulates release of adrenocorticotrophic hormone (ACTH) from the pituitary gland followed by the release of cortisol from the cortices of the adrenal glands. Following exposure to a stressor, it typically takes 20-30 minutes for cortisol levels to reach their peaks (De Kloet, 1991), and multiple negative feedback mechanisms inhibit further production of CRH and ACTH (Gunnar, Bruce, & Hickman, 2001). Cortisol rises in situations associated with physical and psychological stressors, including inflammatory immune responses, heat or cold stress, novelty or uncertainty, social conflict, negative emotions and feelings of threat or loss of control (Kirschbaum & Hellhammer, 1994).

SES and Stress System Activity

In the developed world, higher SES children have lower cortisol concentrations than lower SES children (Essex, Klein, Cho, & Kalin, 2002; Evans & Kim, 2007; Evans & English, 2002; Lupien, King, Meaney, & McEwen, 2000; Lupien, King, Meaney, & McEwen, 2001). These associations have been confirmed in some, but not all, studies in adults (Cohen, Doyle, & Baum, 2006; Dowd & Goldman, 2006). To our knowledge, only two studies have examined economic background and how it relates to stress response in children in a developing country. One study (Flinn & England, 1997, 2003) examined generally low SES children in Dominica. Within the range of economic circumstances they examined, there was no evidence that material benefits (improved housing or diet) were associated with salivary cortisol levels. They did find, though, that children from unstable family backgrounds had higher cortisol levels than did children from stable family homes. The other study set in Nepal measured morning cortisol and a composite index of physiologic functioning — allostatic load, created using cortisol and cardiovascular variables. The physiologic measures differed according to context in which a child lived (e.g, homeless, squatter, village or school) (Worthman & Panter-Brick,

2008); boys who were in school showed a healthier stress profile than those who were squatters or those who were homeless.

Thus, in spite of the small handful of studies that have shown that lower SES is linked with higher and/or dysregulated cortisol, there remains a large research gap relating to whether and how poverty and cortisol are related in young children. Most critically, none of the studies that have examined socioeconomic status and activity of the HPA axis in children or, for that matter, in adults have randomly assigned participants to their economic conditions. Normally, this would not be possible and we are left with correlational findings where the direction of effect and the potential mediators of the association cannot be wholly examined. Thus, the primary goal of this study was to examine the association between income and children's cortisol levels using participants drawn from a large scale, randomized poverty alleviation intervention. This sample allowed examination of the causal relation between income and children's cortisol levels. In addition, because few of the studies of SES and cortisol have examined children living in extreme poverty, the present study extends research on poverty and children's HPA axis activity to children experiencing the type of extreme poverty that is prevalent in many developing countries.

Maternal Depression and Low SES

The studies cited above that have found that lower SES is associated with higher cortisol levels in children have all also reported that the effects of lower SES may be *mediated*, at least in part, by maternal depressive symptoms (Bradley & Corwyn, 2002). Maternal depression is associated with lower income and greater income insecurity in both the developed and developing world (Hadley & Patil, 2008; Pascoe, Stolfi, & Ormond, 2006). When parental care is poor or frequently disrupted during early development, increased levels of cortisol are released in response to threat as measured later in life (Meaney & Szyf, 2005). Depressed mothers tend to provide less sensitive and responsive care to their infants and young children, particularly in the first year of life (Dawson & Ashman, 2000). Further, children of depressed mothers are often less able to meet their children's age-appropriate and stage-salient needs (Arcenio, Sesin, & Siegel, 2004).

If maternal depression serves as an important mediator of the impact of SES on children's cortisol levels, then within the context of a poverty-alleviation intervention study, this would argue for examination of maternal depression as a moderator variable. That is, if under the no-treatment condition children of mothers with high depressive symptoms show the most dysregulated pattern of cortisol production, then under the treatment condition, children of depressed mothers may show the greatest impact on HPA activity. There are also other reasons that maternal depression might moderate child HPA axis activity (Goodman & Gotlib, 1999). Depressed mothers may pass genes to their children that increase the reactivity of the axis to the conditions (pathogens, food insecurity) associated with poverty (Goodman, 2003). Similarly, they may expose their infants to elevated stress hormones during prenatal development, exposures that may increase the sensitivity of the HPA system to postnatal conditions. Thus, using a construct introduced by Boyce and Ellis (2005), the offspring of depressed mothers may be more sensitive to context, showing larger differences than the offspring of non-depressed mothers in response to better or worse family-level factors.

However, there is also a substantial animal and human literature indicating that when parental care is actively supportive, offspring can be relatively *buffered* from the impact of a variety of stressful events (see review, see Gunnar & Donzella, 2002). This process — maternal or parental buffering — reflects the capacity of a supportive adult to prevent or reduce elevations in cortisol, perhaps through stimulation of “anti-stress” hormones such as oxytocin (e.g., Carter, 1998). A responsive and high-functioning mother, however, may also regulate stress

experiences in her offspring through active management of the environment (Leiferman, 2002; McLennan & Kotelchuck, 2000).

Previously, among 2.5- to 6-year old children growing up in urban poverty in Mexico, we reported that among offspring of depressed relative to non-depressed mothers, maternal depression did not affect baseline cortisol levels, but did affect salivary cortisol response to the same mild stressor used in this report (Fernald, Burke, & Gunnar, 2008). Unexpectedly, rather than maternal depression being associated with increased cortisol reactivity, it was associated with a blunted cortisol response. While the blunted response was unanticipated, it may have reflected the impact of chronic stress down-regulating activity of this system (see Miller, Chen, & Zhou, 2007). Thus, there are both theoretical and empirical data suggesting that maternal depression might amplify the effects of poverty on the developing HPA axis.

Objectives of Current Study

The present study uniquely contributes to the literature by using a planned quasi-experimental design to test the impact of *Oportunidades* — a large-scale, poverty-alleviation program — on salivary cortisol concentrations in two to six year old children in rural areas. The goals of the study were to provide an empirical test of the association between the economic conditions of early life rearing and child cortisol levels through examining the impact of family-level participation in *Oportunidades*. Based on previous evidence of a negative association between SES and children's cortisol levels, we hypothesized that children whose families participated in the program would have lower baseline cortisol levels and clearer evidence of a modulated (i.e. rise and return to baseline) cortisol response to the psychosocial stressor task. The less modulated response might include either failure to return to baseline within the sampling period and/or failure to mount a significant response to the stressor tasks. Both patterns of hyper- and hypo-responsiveness of the HPA axis have been observed in response to chronic stress (Fries, Hesse, Hellhammer, & Hellhammer, 2005; Heim, Ehler, & Hellhammer, 2000).

A second objective was to examine whether maternal depression moderated the response to program participation. This objective was supported by previous research showing that child's cortisol responses are influenced by parental stress (e.g. due to marital conflict) (Flinn, 2006) and maternal depression (Ashman, Dawson, Panagiotides, Yamada, & Wilkinson, 2002; Essex et al., 2002; Lupien et al., 2000). During the first years of life, a child's relationship with his or her primary caregiver serves as a significant regulator of reactivity of the HPA axis (Gunnar & Donzella, 2002) and we expected that disturbances in this association due to high maternal depressive symptoms might moderate the impact of the poverty alleviation program on children's cortisol levels.

We chose preschool-aged children for this analysis because alterations in activity of the HPA axis from birth through the early years of life have been hypothesized to affect rapidly developing neural systems involved in emotion- and behavior-regulation (Sullivan & Gratton, 2002).

Methods

Intervention

Oportunidades—a large scale conditional cash transfer program — began in 1997 and was designed to relieve extreme poverty in Mexico; it has been used as a model for initiatives throughout the world. Unrestricted cash payments — typically worth about 20 to 30 percent of household income — were distributed by the program only if children, pregnant women, lactating women, and other family members complied with a series of requirements, including prenatal care, well baby care and immunization, nutrition monitoring and supplementation,

and participation in educational programs regarding health, hygiene and nutrition; program details have been reported previously (Fernald, Gertler, & Neufeld, 2008). The study was approved by the Committee of Ethics in Human Research of the Instituto Nacional de Salud Publica (Mexico's National Institute of Public Health), and by the Center for the Protection of Human Subjects at the University of California at Berkeley; signed consent was obtained from all participants.

Selection of Intervention and Comparison Groups

At its inception, *Oportunidades* determined household eligibility in two stages, first by identifying low-income communities and then by choosing low-income households within those communities (Behrman & Todd, 1999a, 1999b). On average, 78% of the households in selected communities were classified as eligible for program benefits, and 97% of these households enrolled in the program (Behrman & Todd, 1999a, 1999b). Once enrolled, households received benefits for a minimum of three-years conditional on meeting the program requirements; new households were not able to enroll until the next certification period. In 2003, a new comparison group — that had not yet been enrolled in *Oportunidades* — was added as part of a 5-year follow-up survey of the original communities. The same criteria used to select the original communities, but now based on data from the 2000 census, were used to select communities that most closely matched the original communities using propensity score matching methods (Todd, 2004). Once the communities were selected, the same criteria were used as in the selection of the original group to select households within each of these communities.

Sample

The analysis reported here was conducted on a subset of both the intervention and comparison families who had salivary cortisol data. All families lived in communities in rural (defined as towns with < 2,500 inhabitants) Mexico and had incomes < 20th percentile of all Mexican households. For the inclusion in this survey, at least one child between 2 and 6 years of age had to be available at the time of testing. Out of the seven states included in the evaluation, three were randomly chosen to be included in the assessment of stress outcomes in children. In these states, households were randomly chosen to participate in the analysis of stress outcomes, and the oldest child in the selected households between 2 and 6 years of age was selected to serve as the target subject. This was done to weight the analyses towards children who had had longer periods of program participation. Children were not assessed if they were obviously sick (e.g. had a fever, were coughing severely). The final sample consisted of 554 children from the intervention condition and 762 children in the comparison condition.

Data Collection

All participating households were visited without prior appointment by a team of health professionals who conducted an interview with each mother lasting approximately one hour and assessed the children with cognitive tests. Assessments were conducted at any time throughout the day from 0800 to 1600, and included the measures of several measures of physical and mental health.

For the purposes of this report, the primary child outcome measures were salivary cortisol levels and response to a compound stressor: the unexpected arrival of the team of assessors and cognitive testing which required interaction with one of these “strangers”. Researchers arrived unannounced and spent the first 5 minutes primarily in conversation with the mother. One researcher then attempted to engage the child and establish rapport. Following this brief period, the researcher then administered 25–40 minutes of standardized cognitive tasks to assess language and cognitive competence. On average, studies using cognitive testing without a public evaluative component generally fail to evoke an average increase in cortisol when

administered to adults (Dickerson & Kemeny, 2004). An unexpected home visit with strangers is a face valid stressor, encompassing recognized stressful components such as uncontrollability, unpredictability, and social evaluation (Dickerson & Kemeny, 2004), which has been used to provoke stress responses in other studies of children (Kagan, Reznick, & Snidman, 1987; Klimes-Dougan, Hastings, Granger, Usher, & Zahn-Waxler, 2001).

Measures

Salivary cortisol—Saliva samples (100-200 μ l) were collected at three time points from all children during testing in order to reflect the activity of the HPA system. Although some recent studies recommend collecting as many as 15 samples on one day in order to adequately assess HPA axis activity (Hruschka, Kohrt, & Worthman, 2005), a minimum of two samples has been reported to be sufficient to capture a cortisol “response” (Kirschbaum & Hellhammer, 1994). Due to budgetary and time limitations, and previous positive experience with a response protocol (e.g. Fernald & Grantham-McGregor, 1998, 2002; Fernald, Grantham-McGregor, Costello, & Manandhar, 2003) we chose to collect three samples. The first sample was a baseline measure taken approximately 5 minutes after the interview team arrived to the home. This first sample (Time 1) was designed to reflect the activation of the HPA axis before the team’s arrival in the home, given the approximate 20-25 minute time delay between cortisol secretion and subsequent salivary cortisol levels (Kirschbaum & Hellhammer, 1989). We should note, however, that this measure might not reflect activity prior to the research team’s arrival in the village as it seems likely that word of the team’s arrival could have spread quickly from home to home.

Two subsequent cortisol samples were collected 25 (Time 2) and 50 (Time 3) minutes after the first sample was collected. All samples were collected in sterile collection tubes (Salivettes, Sarstedt: Newton, NC) and kept at room temperature for a period no greater than one week. To determine whether room temperature storage would affect results, 10 individuals provided large samples of saliva that were each aliquoted into 4 vials. One vial was immediately frozen at -20°C . The others were stored in a hot house for 1, 4, and 8 weeks prior to assay. There were no significant differences as a function of type and duration of storage. In order to determine the cortisol concentration in the saliva for the study reported here, the samples were assayed in the Biochemical laboratory in the Psychobiology Department, at the University of Trier. After collection in the field, all samples were shipped by express mail to the laboratory where they were stored at -20°C until analysis. After thawing, the samples were assayed in duplicate analyses using a standard time-resolved fluorescence immunoassay. Cortisol levels were determined employing a competitive solid phase time-resolved fluorescence immunoassay with fluoremeric end point detection (DELFI). With a computer-controlled program, a standard curve was generated and the cortisol concentrations of the samples were calculated. Synthetic saliva mixed with cortisol in a range from 0-100 nmol/l served as standards. The intra-assay coefficient of variation was between 3.3% and 4.7% and the inter-assay coefficient of variation was less than 5%. To meet statistical normality assumptions, all salivary cortisol data were logarithmically transformed.

Maternal depressive symptoms—Maternal depressive symptoms were assessed using the Spanish version of the CES-D, a 20-item questionnaire designed to assess intensity of depressive symptoms in the general population (Radloff, 1977); Cronbach’s alpha for the total CES-D score was 0.82. The CES-D has been validated for use in Spanish-speaking populations (Masten, Caldwell-Colbert, Alcala, & Mijares, 1986; Salgado de Snyder & Maldonado, 1994). Scores range from 0 to 60, with a cutoff score in the United States of 16 indicating high risk of clinical depression, but a higher cut-off score has been proposed for adult Mexican women in rural areas (Salgado de Snyder & Maldonado, 1994). In our sample the median CES-D score was 15 (mean = 15.8), and we used the median to group our mothers into “high” versus

“low” depressive symptoms groups. We then created an interaction term with maternal depression and *Oportunidades* program participation to investigate the potential moderating role of depression (Baron & Kenny, 1986).

Additional maternal variables—Educational attainment of the mother was assessed and was included in the model as two dummy variables (some elementary school; some secondary school) in comparison to “no schooling” to account for potentially non-linear effects. Mother’s age was also included in the model.

Household-level variables—Ethnicity of the mother was included as a covariate (1= “indigenous language spoken or all of the time”). Household size was obtained by asking the respondent how many people slept and ate together within the same household. Two proxy measures of household SES were generated: one CE summary measure of household assets (car, van, refrigerator, blender, television, gas heater, boiler, radio, stereo, video cassette recorder, washing machine and fan) and the other of housing quality (quality of roof, wall and floor, number of rooms, presence of indoor bathroom, and presence of indoor electricity). The housing and asset measurements were obtained in a baseline survey that occurred in 1997 in a sub-set (about two-thirds) of the same households. These variables have been shown to provide good estimations of SES in contexts of poverty (Moser, 1998). Principal components analysis was used to summarize the asset variables into one measure and the housing variables into another measure (Falkingham & Namazie, 2002). In both cases, the first principal component was retained (Filmer & Pritchett, 2001). To maintain sample size, data for housing and assets were imputed by community for the households that had not been assessed in 1997, and checks for robustness were administered to ensure that there were no differences between those households with the data and those without.

Community-level variables—A survey of the characteristics of the communities in which the participants lived was conducted through direct observations and interviews with key informants. The community variables collected were: presence of electricity, drainage, street lighting, a preschool, a primary school, a high school, a health center, a mobile health clinic, a formal credit union and an informal credit union.

Analysis Plan

All data were analyzed using multi-level modeling procedures with STATA 9.2. Multi-level analytic techniques account for the hierarchical, nested structure of the data (in this case, 3 observations of cortisol are nested within each individual), allow for modeling the non-independence of residuals, and distinguish between fixed and random effects (Hruschka et al., 2005). To address the hierarchical structure of the data, two levels of equations were estimated (Raudenbush & Bryk, 2002). On the first level, we estimated each individual’s baseline cortisol level (e.g. intercepts) and cortisol reactivity (e.g. slopes). On the second level, we estimated equations to predict differences in level-1 intercepts and slopes from level-2 predictors such as hours since awakening, gender, age, and *Oportunidades* program participation (0=no exposure to the program, 1=exposure to the program).

Cortisol follows a diurnal rhythm, with higher levels after awakening and lower levels later in the day. Time of day as well as time of awakening are independent predictors of cortisol responsivity in adults (Federenko, Wust, Hellhammer, Dechoux, Kumsta, & Kirschbaum, 2004) and children (Flinn & England, 1997). Thus, we created an individual-level covariate (“time since awakening”) that incorporates both sources of variability, which was also centered at its grand mean. All interactions described below were generated and probed according to standard procedures (Aiken & West, 1991). All models described below included two indicator

variables for state, because the survey took place in three states. Robust standard errors were clustered at the individual level.

Differences in salivary cortisol between intervention and comparison children

—Our first hypothesis addressed whether household program participation would be associated with differences in baseline cortisol levels. To test this hypothesis, we generated the following model:

$$\text{CHILD CORT} = \beta_0 + \beta_1 \text{ OPORTUNIDADES} + \beta_2 \text{ TIME SINCE AWAKENING} + \beta_3 \text{ AGE} + \beta_4 \text{ GENDER} + \beta_5 \text{ TIME 2} + \beta_6 \text{ TIME 3} + \beta_7 \text{ COVARIATES} + D\varepsilon$$

β_0 is the estimated intercept representing the average individual's baseline level of cortisol (Time 1), β_1 is the estimated effect of *Oportunidades* program participation on cortisol at Time 1, β_2 is the estimated effect of time since awakening on cortisol at Time 1, β_3 is the estimated effect of child's age on cortisol at Time 1, β_4 is the estimated effect of child's gender on cortisol at Time 1, β_5 is an indicator variable for Time 2 allowing us to assess reactivity from Time 1 to Time 2, β_6 is an indicator variable allowing us to compare salivary cortisol concentration at Time 3 with Time 1, and also to calculate recovery from Time 2 to Time 3 and β_7 represents a set of additional covariates, including demographic and socio-economic information (mother's age, mother's educational attainment, ethnicity of the head of the household, household size, a housing factor and an asset factor), and a composite factor representing community characteristics.

Differences between intervention and comparison children in salivary cortisol reactivity or change over the course of the testing period—Our second hypothesis addressed whether program participation would be associated with different cortisol responsivity or change over the three time points of salivary cortisol collection. To test this hypothesis, we used the following model:

$$\text{CHILD CORT}_{ti} = \beta_0 + \beta_1 \text{ OPORTUNIDADES} + \beta_2 \text{ TIME SINCE AWAKENING} + \beta_3 \text{ AGE} + \beta_4 \text{ GENDER} + \beta_5 \text{ TIME 2} + \beta_6 \text{ TIME 3} + \beta_7 \text{ TIME 2} * \text{ OPORTUNIDADES} + \beta_8 \text{ TIME 3} * \text{ OPORTUNIDADES} + \beta_9 \text{ COVARIATES} + \varepsilon$$

In this model, compared with the model described above, we added β_7 , which is the estimated interaction of *Oportunidades* program participation and response at Time 2, and β_8 , which is the estimated interaction of *Oportunidades* program participation and recovery by Time 3. The coefficients were interpreted as follows, using post-estimation linear combinations of estimators. Change from Time 1 to Time 2 was interpreted as β_5 in comparison children and $\beta_5 + \beta_7$ in intervention children; the difference between the groups was β_7 . Change from Time 2 to Time 3 was interpreted as $\beta_6 - \beta_5$ in comparison children and $\beta_6 + \beta_8 - \beta_7 - \beta_5$ in intervention children; the difference between the groups was $\beta_8 - \beta_7$.

Modifying effect of maternal depressive symptoms on differences between intervention and comparison groups—Our third hypothesis addressed whether the effect of *Oportunidades* program participation was moderated by maternal depression. To test this hypothesis, we used the following equation:

$$\text{CHILD CORT} = \beta_0 + \beta_1 \text{ OPORTUNIDADES} + \beta_2 \text{ TIME SINCE AWAKENING} + \beta_3 \text{ AGE} + \beta_4 \text{ GENDER} + \beta_5 \text{ TIME 2} + \beta_6 \text{ TIME 3} + \beta_7 \text{ MATERNAL DEPRESSION} + \beta_8 \text{ OPORTUNIDADES} * \text{ MATERNAL DEPRESSION} + \beta_9 \text{ COVARIATES} + \varepsilon$$

Then, in order to assess differences in reactivity and recovery by maternal depressive symptoms, we added $\beta_{10} \text{ TIME 2} * \text{ OPORTUNIDADES}$ and $\beta_{11} \text{ TIME 3} *$

OPORTUNIDADES to the model, as well as two three-way interactions representing maternal depression x program participation x salivary cortisol reactivity.

Results

Sample Characteristics

A total of 1197 children aged 2-6 y from rural areas of Mexico participated in the study. The mean age of the sample was 49.0 ($SD = 13.9$) months. Characteristics of intervention and comparison households are presented in Table 1. The groups were well matched according age, gender and time since wake-up of the participants. Other characteristics were similar including maternal depressive symptoms, the proportion of the sample that had received some primary education, and the index of community-level variables. However, in spite of the fact that the groups had been selected to be socio-economically similar, differences between the groups were apparent. Households that were *Oportunidades* program beneficiaries were more likely to be indigenous, to be larger, and to have lower housing and asset indices.

Oportunidades Program Participation and Salivary Cortisol

Mean cortisol level—Arithmetic and geometric mean and median salivary cortisol concentrations at all three time points are presented in Table 2. In a repeated measures analysis children in the intervention group had lower salivary cortisol concentrations (log-transformed) when adjusting for child age, gender, and time since awakening ($\beta = -0.12$ (95% CI: $-0.19, -0.05$), $p < 0.001$) (Table 3, Model 1); the significance of the coefficient was not altered with the inclusion of several covariates (Table 3, Model 3). Predicted means across the three time points are presented in Figure 1. Time since wake-up contributed significantly to the model, but child age and sex did not.

Change in cortisol in response to testing—Children in both groups mounted a cortisol response to being visited by a team of unknown interviewers, showing a significant increase between Time 1 and Time 2 ($\beta = 0.11$ (95% CI: $0.05, 0.17$), $p=0.001$) (Model 2, Table 3). However, the magnitude of the response did not differ significantly between intervention and comparison groups, either in terms of the rise from Time 1 to Time 2 or the decrease from Time 2 to Time 3. The magnitude of decrease from Time 2 to Time 3 was $\beta = -0.11$ (95% CI: $-0.17, -0.05$, $p < 0.0001$ (data not shown).

Maternal Depression and Cortisol

There was no main effect of maternal depression on children's mean cortisol level. However, maternal depression was a significant moderator of the association between program participation and salivary cortisol ($\beta = -0.19$ (95% CI: $-0.34, -0.05$), $p=0.009$) (Model 4, Table 3), and this association did not change with the inclusion of covariates (Model 5, Table 3). Specifically, there was a large and significant *Oportunidades* program effect of lowering cortisol in children of mothers with high depressive symptoms but not in children of mothers with low depressive symptomatology (Figure 2). There was a significant difference in salivary cortisol levels between the intervention and comparison group for children of mothers in the high depressive symptoms group. For children of mothers in the low depressive symptoms group the mean for the intervention group was lower than that of the comparison group; however, the difference was not significant.

Discussion

Previous studies have yielded evidence that socioeconomic status is negatively associated with salivary cortisol levels in children. Nonetheless, while growing up under conditions of poverty has been hypothesized to cause changes in HPA axis activity, this hypothesis has never been

tested experimentally. The present study provides the first evidence in support of this causal hypothesis. Children who were born into families who had been assigned to the *Oportunidades*, poverty-alleviation program, and thus had spent all or most of their early years in the program, had lower salivary cortisol levels than did children whose families were not given the opportunity to take part in the program. Although animal models of early life stress would predict an impact on HPA reactivity and negative feedback regulation, we found no difference between groups in the pattern of cortisol change to arrival and interaction with the unfamiliar testers because both groups responded similarly. Finally, we had hypothesized that the main effect of program participation might be qualified by maternal depressive symptomatology, and part of our hypothesis was confirmed because the impact of program participation on baseline salivary cortisol concentration was significant only in children of mothers with high depressive symptoms. However, there was no moderating impact of maternal depression on cortisol reactivity or recovery.

Animal work on early experiences implicates parental care as critical in shaping the development of this neuroendocrine system (Meaney & Szyf, 2005). Prior studies of economic stress have found that maternal depressive symptoms mediated associations between family economic conditions and child cortisol levels (e.g. Essex et al., 2002). These findings were interpreted as consistent with the animal data because maternal depression often impairs the mother's ability to provide sensitive and supportive care (e.g. Dawson & Ashman, 2000). In a previous study examining cortisol reactivity in children growing up in urban poverty in Mexico, we noted that the high reported depressive symptoms among the mothers were associated with blunted cortisol reactivity among their children, although maternal depression had no impact on baseline cortisol levels (Fernald, Burke, & Gunnar, 2008). Consistent with the findings in the developed world, we viewed our findings as potential evidence that poverty impacts the child's HPA axis, at least in part through affecting the mother's capacity to provide supportive, buffering care, which are capacities impaired by maternal depression.

Although the *Oportunidades* program provided minimal training in parenting, it is conceivable that participation in the program reduced maternal financial anxieties and allowed the mother to focus more attention on supportive care of her young children. If so, we hypothesized that we would observe the strongest program effects for children whose mothers were high in depressive symptoms. Specifically, we expected that for all children, program participation would reduce baseline cortisol levels and produce more modulated (i.e. response and return to baseline) cortisol reactivity; but we anticipated larger effects for children of depressed mothers whose HPA activity we expected would be more dysregulated for the control children. The results were not quite as anticipated. We did not observe any evidence of a blunted or otherwise dysregulated cortisol stress response among the control children of depressed mothers. We did, however, find that among the control group, cortisol levels were higher for children of depressed versus non-depressed mothers, replicating several reports about children in developed countries. We also found that the strongest impact of program participation on cortisol levels was among the children of depressed mothers, as predicted. For children of depressed mothers, program participation reduced cortisol levels by about a half.

We should caution, however, that there is an alternative explanation for the difference in baseline between the control and *Oportunidades* treatment families. Although we used the initial cortisol sample as a baseline measure because it was taken before the children could have responded to the presence of the testers in their homes, it is possible that news of the teams' arrival in the villages reached the home before the testers arrived. As the team would be a greater novelty in the control than in the treatment villages, it is possible that the higher baselines for the control versus the treatment children actually reflected a cortisol response to the increased novelty of the testing team being in the village. While we cannot rule out this possibility, it is not consistent with the comparable increases to testing seen between the

treatment and control children. If cortisol was already elevated when the team arrived at the child's door, then negative feedback control of the axis would have reduced further increases in response and we would have found both higher baselines and blunted cortisol responses to testing. The most parsimonious explanation of the finding is that between the treatment and control children there was a significant baseline difference.

There are several interpretations of these interactions between the *Oportunidades* program and maternal depression. First, non-depressed mothers may have been able to buffer or compensate for the impact of poverty on early development of the HPA axis. Second, while speculative, the fact that we observed such a strong effect among the children of depressed mothers might reflect differences in temperament between these children and those whose mothers were not high on depressive symptoms. Specifically, genetic similarities between mother and child may have results in the children of depressed mothers being temperamentally more sensitive to context. Boyce and Ellis (2005) argue that children who are temperamentally more sensitive and reactive are more negatively impacted by adverse circumstances, and under developmentally supportive conditions actually function *better* than less temperamentally reactive children. Either of these explanations would predict larger differences between the children of depressed and non-depressed mothers under the no treatment condition.

We should also note, however, that there is a third explanation for these findings that casts lower cortisol levels as a reflection of chronic stress. In rats, chronic intraventricular infusions of glucocorticoids decrease CRH production in the hypothalamus (Makino, Gold, & Schulkin, 1994). Decreased ACTH production to CRH challenge has been observed in individuals exposed to early life stress (Heim, Newport, Bonsall, Miller, & Nemeroff, 2001). These types of impacts may explain why cortisol levels may be normal or sub-normal in individuals exposed to chronic stress (e.g. Miller et al., 2007). Depressed mothers exhibit difficulties in responding to the needs and signals of their children; adding the demands of program participation might conceivably have exacerbated these difficulties, resulting in children of depressed mothers being more chronically stressed if their family was in the program when compared with those who were not. This explanation seems unlikely because children of both depressed and non-depressed mothers were able to mount a cortisol response to the arrival of the testing team. Hypocortisolism due to down-regulation of the axis in response to chronic stress is reflected not only in a lower baseline, but also blunted cortisol responses to stressors.

Indeed, although we anticipated that the program might impact the magnitude of cortisol response to the psychosocial stressor of the experimenter's arrival and testing of the children, no effects of program or maternal depression were found for the shape of the cortisol response. In both groups, cortisol increased from Time 1 to Time 2 and then declined at Time 3 back to the Time 1 level. This pattern of response clearly indicates that the challenge of the visitor's arrival in the home was a significant stressor for these children; thus our failure to obtain reactivity differences cannot be attributed to use of an ineffective stressor stimulus. It might, however, be due to the stimulus being too mild a challenge to reveal differences between intervention and comparison children. The increase in cortisol was small, less than 18% on average for the two groups combined. While this is not atypically small for mild stressors such as the one used in this report, it may have been too mild a reaction to reveal differences between groups.

There were several limitations to this study that should be noted. First, we examined only a subsample of children from *Oportunidades*, and thus we cannot say for certain whether this sample was wholly representative of the larger sample. A related limitation is that we do not know why the later intervention groups were not eligible in the initial rounds of enrollment into the *Oportunidades* program, in spite of the fact that the program used a quasi-randomized experiment. Nonetheless, we controlled for a wide array of household-level and community-

level variables, and found these to have no impact on the results. Second, we did not control for — nor did we have data available for — several variables that could have confounded salivary cortisol results, such as food eaten, caffeine intake, health problems, individual-level early life stress including exposure to marital discord, or current post-traumatic stress disorder. It is possible that one or a combination of these variables may have influenced the results and, as noted for health and early life stress, may have been important in mediating the impact of this type of cash transfer program on child stress biology. A third limitation to the interpretation of our results is that we measured salivary cortisol, which is the end product of a complex, distributed stress neurobiology and only provides insight into one part of the body's stress system (Granger & Kivlighan, 2003). Thus, we are only able to comment on the concentrations of cortisol, and can only speculate about the central neural mechanisms that may explain group differences in cortisol levels. A fourth limitation is that we collected only three measurements of cortisol across the day; in an ideal study we would have been able to collect more samples from each individual child and we could have tested the children and collected the samples at a standardized time (e.g. first thing in the morning). Unfortunately, neither of these conditions was possible due to constraints of this large quasi-randomized trial.

In spite of these limitations, we are encouraged by many strengths of the study, including the large sample size, the collection of multiple samples per person, control for time of day and time of wake-up, use of a well-standardized and culturally acceptable measure of depressive symptoms, and collection of these measures and outcomes in a previously under-researched sample of low-income families in rural Mexico. Accordingly, these results provide the strongest evidence to date that the economic circumstances of the family impact the child's developing stress system providing one mechanism through which poverty early in life impacts life-course risk for physical and mental health disorders.

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Figure 1. Predicted salivary cortisol (nmol/L) by intervention group across three time points, adjusted for child age, sex and time of since awakening

Figure 2.

Predicted log-transformed salivary cortisol (mean value over three time points) by *Oportunidades* program participation (*Oportunidades* v. Comparison) and maternal depression^a (high v. low symptoms, split at median)

a. Maternal depressive symptoms were assessed using the Spanish version of the CES-D, a 20-item questionnaire designed to assess intensity of depressive symptoms in the general population (Radloff, 1977).

Table 1

Descriptive Characteristics of Sample^a

	<i>Oportunidades</i> ^b Intervention households N=491	Comparison households N=706	P
Child characteristics			
Age, m	49.4 (13.9)	48.4 (14.1)	0.21
Female (%)	48.4%	52.3%	0.17
Time of wake-up	07:42 (48m)	07:30 (54m)	<0.0001
Time of testing	13:58 (3h)	13:36 (2h, 42m)	0.04
Time since wake-up (min), centered at 0	-14.7 (287.8)	-2.6 (310.42)	0.50
Maternal characteristics			
Depressive symptom score ^c	16.4 (9.5)	15.4 (9.4)	0.10
Score above sample median ^d (%)	50.6	46.6	0.18
Age, y	32.3 (7.3)	30.3 (7.9)	<0.0001
Education			
No formal education	13.4%	5.7%	<0.0001
Some primary education	72.7%	68.6%	0.12
Some secondary education	13.8%	25.8%	<0.0001
Household characteristics			
Household ethnicity ^e (% indigenous)	17.5%	5.9%	<0.0001
Household size (number of people)	6.3 (2.5)	5.6 (2.4)	<0.0001
Baseline housing index ^f	0.6 (1.4)	1.0 (1.3)	<0.0001
Dirt floor in home (%)	45.1%	35.0%	0.003
Bathroom in home (%)	39.1%	46.6%	0.03
Electricity in home (%)	81.8%	89.4%	0.002
Quality of walls (range 1-3)	2.2 (0.8)	2.1 (0.9)	0.001
Quality of roof (range 1-3)	2.1 (0.9)	2.0 (1.0)	0.17
Water available on land (%)	50.7%	63.7%	<0.0001
Water available in the home (%)	10.7%	20.6%	<0.0001
Baseline asset index ^g	0.6 (1.8)	1.3 (2.0)	<0.0001
Car (%)	8.4%	21.2%	<0.0001
Van (%)	6.9%	9.3%	0.14
Refrigerator (%)	30.3%	47.3%	<0.0001
Blender (%)	59.0%	68.3%	0.001
Television (%)	68.0%	75.9%	0.003
Gas heater (%)	50.5%	60.8%	<0.0001
Radio (%)	73.5%	80.9%	0.003
Stereo (%)	7.2%	7.8%	0.69
Video (%)	4.2%	6.6%	0.08
Washing machine (%)	4.2%	4.8%	0.66
Fan (%)	1.8%	6.8%	<0.0001
Community characteristics			
Community index ^h	0.04 (1.0)	0.09 (0.7)	0.27
Majority indigenous population (%)	23.0%	11.9%	<0.0001
Electricity available (%)	84.7%	81.9%	0.20
Drainage available (%)	12.6%	30.7%	<0.0001
Telephone lines available (%)	84.9%	83.1%	0.41
Preschool present (%)	97.6%	100%	<0.0001
Primary school present (%)	100%	100%	N/A
High school present (%)	5.1%	2.5%	0.02
Permanent health center (%)	73.1%	70.8%	0.39
Mobile health center (%)	80.7%	75.2%	0.03
Formal credit union (%)	2.6%	1.3%	0.08
Informal credit union (%)	13.6%	15.3%	0.43
State characteristics			
Hidalgo (central, NE of Mexico City)	20.4%	6.1%	<0.0001
Michoacan (central, W of Mexico City)	55.7%	51.7%	0.16
Querétaro (central, N of Mexico City)	23.9%	42.2%	<0.0001

^a Mean (SD) presented unless otherwise noted. Data collected in 2003 unless otherwise noted.

^b *Oportunidades* intervention is large-scale poverty alleviation program (conditional cash transfer program).

^c Maternal depressive symptoms were assessed using the Spanish version of the CES-D (Center for Epidemiologic Studies-Depression Scale), a 20-item questionnaire designed to assess intensity of depressive symptoms in the general population (Radloff, 1977).

^d Sample median was 15 and mean was 15.8.

^e Indigenous status obtained by asking mother what language was predominantly spoken at home.

^fBaseline housing index constructed using principal components analysis of the following variables (information collected in 1997): quality of roof, wall and floor, number of rooms, presence of indoor bathroom, and presence of indoor electricity.

^gBaseline asset index constructed using principal components analysis of the following variables (information collected in 1997): car, van, refrigerator, blender, television, gas heater, boiler, radio, stereo, video cassette recorder, washing machine and fan.

^hCommunity index using presence of electricity, drainage, street lighting, a preschool, a primary school, a high school, a health center, a mobile health clinic, a formal credit union and an informal credit union; data collected in 2003.

Mean, geometric mean and median cortisol concentrations (nmol/L)^a in 2.5 – 5 year old children from *Oportunidades*^b intervention (n=491) and comparison households (n=706) in rural Mexico.

Table 2

Time	Mean (SD)		Geometric mean (95% CI)		Median (IQR)	
	Intervention	Comparison	Intervention	Comparison	Intervention	Comparison
1	2.5 (2.7)	2.8 (3.0)	1.7 (1.6, 1.8)	2.0 (2.6, 3.1)	1.7 (0.9, 3.2)	2.0 (1.1, 3.5)
2	2.9 (2.9)	2.9 (2.4)	2.0 (1.8, 2.1)	2.1 (2.0, 2.3)	2.0 (1.1, 3.7)	2.2 (1.3, 3.7)
3	2.4 (2.3)	2.8 (2.6)	1.7 (1.6, 1.8)	2.0 (1.8, 2.1)	1.7 (1.0, 3.0)	1.9 (1.1, 3.6)

^aTime 1 cortisol collected upon arrival at household, Time 2 cortisol specimen collected an average of 25 minutes after baseline; Time 3 was collected 50 minutes after baseline.

^b*Oportunidades* intervention is large-scale poverty alleviation program (conditional cash transfer program).

Table 3

Repeated measures analyses examining impact of *Oportunidades* program participation on salivary cortisol (log-transformed) in young children over three time points representing baseline (Time 1), reactivity (Time 2) and recovery (Time 3) (n=1197)^a, presented in three models^b.

	1: Cortisol level	2: Reactivity	3: Adjusted	4: Depression	5: Adjusted
<i>Oportunidades</i> intervention	-0.12*** (-0.19, -0.05)	-0.12*** (-0.26, -0.05)	-0.13*** (-0.21, -0.06)	-0.03 (-0.34, -0.09)	-0.06 (-0.17, 0.05)
Time since wake-up, centered	-0.00*** (0, 0)	-0.00*** (0, 0)	-0.00*** (0, 0)	-0.00*** (0, 0)	-0.00*** (0, 0)
Child age, m	-0.002 (-0.004, 0.001)	-0.002 (-0.004, 0.001)	-0.001 (-0.004, 0.001)	-0.001 (-0.02, 0)	-0.001 (-0.004, 0.001)
Gender (1=female)	0.05 (-0.02, 0.12)	0.05 (-0.02, 0.12)	0.05 (-0.17, 0.12)	0.06 (-0.01, 0.04)	0.07 ⁺ (-0.01, 0.14)
Time 2 ^c		0.11** (0.05, 0.17)	0.12*** (0.05, 0.18)		0.13*** (0.07, 0.19)
Time 3 ^c		-0.01 (-0.07, 0.05)	-0.01 (-0.07, 0.05)		0 (-0.06, 0.06)
Mother's age, yrs			-0.003 (-0.008, 0.001)		-0.001 (-0.006, 0.004)
Mother's ed., some primary ^d			-0.13 (-0.27, 0.001)		-0.11 (-0.26, 0.04)
Mother's ed., some secondary ^d			-0.15 (-0.31, 0.003)		-0.13 (-0.30, 0.04)
Ethnicity (1=indigenous)			0.17 (0.04, 0.30)		0.18 (0.04, 0.32)
Household size, number			-0.005 (-0.02, 0.009)		0.02 (-0.15, 0.06)
Housing index ^e (1997)			0.02 (-0.01, 0.06)		-0.004 (-0.02, 0.02)
Asset index ^e (1997)			-0.004 (-0.02, 0.02)		-0.004 (-0.02, 0.02)
Community composite variable ^f			-0.02 (-0.07, 0.28)		-0.02 (-0.06, 0.02)
Maternal depression (CES-D ⁺)				0.06 (-0.03, 0.15)	0.04 (-0.05, 0.14)
<i>Oportunidades</i> x CES-D				-0.19** (-0.34, -0.05)	-0.18* (-0.05, 0.14)

Notes:

^a95% CI in parentheses. State level fixed effects included in all models; clustering at individual level

^bEquation for analyses in first three models is: CHILD CORT = $\beta_0 + \beta_1$ OPORTUNIDADES + β_2 TIME SINCE AWAKENING + β_3 AGE + β_4 GENDER + β_5 TIME 2 + β_6 TIME 3 + β_7 COVARIATES + e. Equation for analyses in fourth and fifth models is: CHILD CORT = $\beta_0 + \beta_1$ OPORTUNIDADES + β_2 TIME SINCE AWAKENING + β_3 AGE + β_4 GENDER + β_5 TIME 2 + β_6 TIME 3 + β_7 MATERNAL DEPRESSION + β_8 OPORTUNIDADES * MATERNAL DEPRESSION + β_9 COVARIATES + e

^cTime 2 cortisol specimen was collected an average of 25 minutes after baseline (Time 1); Time 3 was collected 50 minutes after baseline.

^dMaternal education variables are indicator variables in contrast to "no education" to account for potentially non-linear effects.

^eComposite variables constructed using principal components analysis: assets (car, van, refrigerator, blender, television, gas heater, boiler, radio, stereo, video cassette recorder, washing machine and fan), housing (quality of roof, wall and floor; number of rooms, presence of indoor bathroom, and presence of indoor electricity), community (presence of electricity, drainage, street lighting, a preschool, a primary school, a high school, a health center, a mobile health clinic, a formal health union and an informal credit union)

^fMaternal depressive symptoms were assessed using the Spanish version of the CES-D, a 20-item questionnaire designed to assess intensity of depressive symptoms in the general population (Radloff, 1977).

+ sig at 0.10

* sig at 0.05

** sig at 0.01

*** sig at 0.0001