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Title: Hair cortisol levels, perceived stress and body mass index in women and children living in socioeconomically disadvantaged neighbourhoods: The READI study

Running head: Hair cortisol, perceived stress and BMI

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ABSTRACT

Disadvantaged communities provide adverse psychosocial exposures that have been linked to high levels of stress, and this may provide one explanatory pathway linking socioeconomic disadvantage to obesity. This study used hair cortisol analysis to quantify associations between stress and body mass index (BMI), and between hair cortisol and perceived psychological stress levels, in women and children living in socioeconomically disadvantaged neighbourhoods. Participants were a volunteer sample of 70 women from the Resilience for Eating and Activity Despite Inequality (READI) study, including 30 maternal-child pairs. Women self-reported body weight, height and perceived psychological stress using the Perceived Stress Scale (PSS), and provided hair samples for themselves and their child. Children’s body weight and height were measured. Following extraction, hair cortisol levels were measured using enzyme-linked immunosorbent assay. Multiple linear regression models examined associations between stress and BMI, and between hair cortisol and perceived stress levels in women and children. Women's hair cortisol levels were not associated with their BMI or PSS scores. Women’s PSS scores were positively associated with their BMI (p=0.015). Within maternal-child pairs, mothers and children’s hair cortisol levels were strongly positively associated (p=0.006). Maternal hair cortisol levels and PSS scores were unrelated to their child's zBMI. Children's hair cortisol levels were not associated with their zBMI or with their mother's PSS score. Findings suggest that cortisol-based and perceived psychological measures of stress may be distinct among women and children living in disadvantaged neighbourhoods. Perceived psychological measures may be more important predictors of weight-related risk.
INTRODUCTION

Obesity is among the most pressing public health issues worldwide due to its high prevalence and association with negative physical, psychological and social consequences across the lifecourse (Lim et al., 2012). A strong socioeconomic gradient in obesity exists in developed nations, such that those who are less educated, earn less income, live in disadvantaged neighbourhoods, or are employed in less prestigious occupations face an increased risk (Ellaway et al., 1997; King et al., 2006; Matheson et al., 2008; Robert and Reither, 2004; van Lenthe and Mackenbach, 2002). In Australia for instance, the prevalence of overweight and obesity in 2011-12 among those living in the least disadvantaged areas was 50%, compared with 59% among those living in the most disadvantaged areas (Australian Bureau of Statistics, 2013). It has been predicted that if current Australian trends do not abate, inequalities in weight will widen by 2025, such that there will be a 14 percentage point difference in obesity rates between the most (i.e. tertiary) and least (i.e. secondary only) educated Australian adults (Backholer et al., 2012).

The etiology of obesity is complex, as weight gain is influenced by factors ranging from inherited biological traits to behavioural, social and environmental factors; only some of which operate through their impact on dietary and physical activity behaviours (Government Office for Science, 2007). Accumulating evidence suggests psychosocial stress may be implicated in weight gain through direct biological and behavioural pathways (Moore and Cunningham, 2012; Wardle et al., 2011). Biologically, the stress response is characterised by activation of the hypothalamo-pituitary-adrenal (HPA) axis in an attempt to maintain homeostasis (Bjorntorp, 2001). Activation of the HPA axis invokes increases in circulating cortisol, a glucocorticoid that increases abdominal adiposity and enhances appetite with
prolonged elevation (Bjorntorp, 2001). Behavioural mechanisms linking stress with obesity include increased consumption of energy-dense convenience foods (Tryon et al., 2015; Wardle J and Gibson EL, 2002), use of food for comfort or as a coping strategy (Dallman, 2010; Habhab et al., 2009; Zellner et al., 2006), and reduced participation in physical activity (Mouchacca et al., 2013; Steptoe et al., 1996). Together, these biological and behavioural responses establish a context tending towards weight gain. Children may be particularly susceptible to stress-induced weight gain, as both their own and their parents’ experiences of stress increase children’s risk of unhealthy dietary behaviours and weight gain (Gundersen et al., 2011; Gundersen et al., 2008; Parks et al., 2012; Shankardass et al., 2014). Moreover, evidence indicates that the level of cortisol in hair may be an inherited trait (Fairbanks et al., 2011).

Classic correlates of energy intake and expenditure do not fully explain socioeconomic gradients in obesity (Ball et al., 2002; Croft et al., 1992; Matheson et al., 2008; Pudrovska et al., 2014; Purslow et al., 2008). Given that socioeconomic disadvantage has been linked to high levels of psychosocial stress (Moore and Cunningham, 2012), elevated stress may provide one explanatory pathway linking socioeconomic disadvantage to obesity. Evidence indicates that individuals who reside in disadvantaged neighbourhoods are often embedded within environments in which they are relatively more likely to encounter negative or threatening situations (e.g. crime, disrepair, restricted access to grocery stores and recreational amenities), leading to psychological distress and its associated biological and behavioural perturbations (Burdette and Hill, 2008; Hill et al., 2005; Ross et al., 2000). Moreover these studies show that neighbourhood disadvantage is an independent source of stress, over and above that conferred by individual-level SES and other factors. A large body of evidence indicates bi-directional associations among disadvantage, stress and obesity.
(Burdette and Hill, 2008; Moore and Cunningham, 2012; Schulz et al., 2008), with a smaller number of studies suggesting that stress may mediate associations between disadvantage and obesity (Burdette and Hill, 2008; Moore and Cunningham, 2012; Schulz et al., 2008). Notably, this body of knowledge has been derived almost entirely from studies that have assessed perceived psychological stress levels, with consequent variation in ways in which the concept of stress has been defined and operationalized, and limitations associated with self-reporting. A smaller number of studies have examined these associations using physiological measures of stress such as salivary cortisol, finding that disadvantaged neighbourhood contexts may influence baseline cortisol levels (Chen and Paterson, 2006) and patterns of cortisol secretion over the course of the day independent of individual-level SES (Do et al., 2011). A systematic review that examined associations between physiological measures of cortisol activity (primarily in saliva, urine and blood) and obesity found a general pattern wherein greater abdominal fat was associated with greater responsivity of the HPA axis, although some studies did show underresponsiveness (Incollingo Rodriguez et al., 2015). The analysis of physiological cortisol levels in saliva and blood has important limitations, however, as cortisol levels in these mediums vary widely and systematically over the course of the day, reflect acute exposures up to minutes prior to data collection, are highly sensitive to context, and should ideally be sampled repeatedly over several days (Chan et al., 2014; Do et al., 2011; Fairbanks et al., 2011; Kudielka et al., 2009).

The limitations of extant methods of quantifying stress levels have led investigators to explore the feasibility of using hair cortisol analysis as an alternate or complementary physiological marker of stress (Gow et al., 2010; Incollingo Rodriguez et al., 2015; Russell et al., 2012). The analysis of cortisol levels in hair provides a more objective marker of stress compared to psychological measures, and may be more reproducible than other physiological
measures of stress because it aggregates day-to-day variations in HPA axis activity, thereby reflecting chronic rather than acute and transient stressful exposures (Russell et al., 2012). Moreover, measurement is non-invasive and simple, requiring a single sample of hair. The validity of the technique is supported by studies showing that hair cortisol levels are elevated in patients with known hypercortisolism, and that they correspond with clinical disease course in patients with Cushing’s syndrome and Addison’s disease (Manenschijn et al., 2011; Thomson et al., 2010).

To date, hair cortisol analysis has not been used to examine associations between stress and obesity among individuals living in disadvantaged neighbourhoods who are at high risk of both. Different physiological measures of stress may show different associations with obesity compared to one another, and in comparison to perceived psychological measures. Therefore, the purpose of this study was to use hair cortisol analysis to quantify associations between a physiological indicator of chronic stress levels and body mass index (BMI), and between hair cortisol and perceived psychological stress levels in women and children living in socioeconomically disadvantaged neighbourhoods. We also considered the association between mother and child hair cortisol concentrations to examine the heritability of hair cortisol levels. We hypothesized that the hair cortisol and perceived stress levels of women living in disadvantaged areas would be positively associated with one another, and with their BMI. We expected that maternal and child hair cortisol levels would also show positive associations, and that children’s zBMI would be associated with their own hair cortisol levels and those of their mother.

MATERIALS AND METHODS

Participants and procedures
Data for these analyses were provided in 2012-2013 by women and children participating in the Resilience for Eating and Activity Despite Inequality (READI) study, methods of which have been detailed elsewhere (Ball et al., 2013). The study was approved by the Deakin University Human Research Ethics Committee (HEAG-H 91_2006). Women provided written informed consent for themselves and their child to participate.

To obtain a representative sample of disadvantaged participants, READI used the Socio-Economic Index for Areas (SEIFA) (Australian Bureau of Statistics, 2001) to classify all neighbourhoods (suburbs) in Victoria, Australia, into tertiles of disadvantage. The SEIFA is based on 2001 population census data, and uses a broad definition of socioeconomic disadvantage that reflects individuals’ access to material and social resources, and their ability to participate in society. Areas in the lowest SEIFA tertile were considered disadvantaged, and 40 urban and 40 rural areas were randomly selected from this sampling frame. The electoral roll (voting is compulsory in Australia) was then used to randomly identify 150 women aged 18–45 years from each of these 80 areas (n=11 940; as some included areas had fewer than 150 eligible women). Participants (n=4934; 41% response rate) replied to a postal invitation to complete a questionnaire (baseline; 2007-08). Those who consented to further follow-up and remained eligible to participate in the study (i.e. had not moved outside of the study neighbourhoods) were re-contacted to complete follow-up surveys 3 (2010-11) and 5 years (2012-13) after baseline. In 2012-13, completed surveys were received from 1560 women, of whom 277 also completed a child survey. A random sample of 199 of these women plus all 266 mother-child pairs who had consented to additional follow-up were then invited to take part in a sub-study involving hair cortisol analysis. A total of 116 women consented to take part in the sub-study, 60 of whom also consented on behalf of their child. Women who were pregnant were excluded.
Data collection and analysis

Perceived stress

Women’s perceived stress levels were assessed using the four-item version of the Perceived Stress Scale (PSS), a commonly used and validated measure of the degree to which respondents felt their lives were unpredictable, uncontrollable or overwhelming in the past month (Cohen and Williamson, 1988; Cohen et al., 1983). The specific questions asked were: “During the last month how often have you: (i) felt that you were unable to control the important things in your life?; (ii) felt confident about your ability to handle your personal problems?; (iii) felt that things were going your way?; and (iv) felt difficulties were piling up so high that you could not overcome them?”. Responses options were: never, almost never, sometimes, fairly often, and very often. PSS scores were obtained by reverse coding the positive items (ii & iii), and summing scores for the four items. PSS scores can range from 0 – 16 (MacArthur Foundation), with an overall mean of 4.49 (SD 2.96) in a representative US sample (Cohen and Williamson, 1988). Higher scores indicated greater perceived stress.

Body mass index

Women self-reported height and weight to calculate BMI (kg/m²). Children’s height (to the nearest 0.1 cm) and weight (to the nearest 0.1 kg) were measured without shoes and in light clothing by trained research assistants within 4 months of maternal survey completion using a portable stadiometer and digital scales. Age- and sex-adjusted BMI z-scores (zBMI) were calculated for children based on the Centers for Disease Control reference population (Kuczmarski et al., 2000). Children were classified as normal weight, overweight or obese using cut-off points established by Cole et al (Cole et al., 2000).
**Sociodemographic factors**

Women reported their age, country of birth, smoking status (never, former, current occasional, current regular), highest education level (low: did not complete year 12, medium: completed year 12 or equivalent, high: tertiary qualification), marital status (married/de facto, previously married, never married), number of children living in the home (0, 1, 2, ≥ 3), employment status (working full time, working part-time, not currently in paid employment), and household income (low: $0–699 per week, medium: $700–1499 per week, high: $1500+ per week, undisclosed), and whether they had dyed their hair (yes, no).

**Hair samples**

*Hair sample collection:* Consenting women were sent a hair sampling kit. The kit contained instructions on how to cut approximately 100 strands of hair as close to the scalp as possible from the posterior vertex position of the head for themselves and their child. This area of the scalp was selected because it has the lowest coefficient of variation (15.6%) compared to hair sampled from other areas (30.5%) (Sauve et al., 2007). Women then taped the hair sample to a paper slip provided with the scalp end marked. Samples were returned in sealed postage-paid envelopes, as per standard procedures (Gow et al., 2010).

A total of 102 hair samples were received from women (22% of those invited to participate) and 54 from children (20% of those invited to participate). Excluding participants whose hair samples were unusable (too few or too short strands, or did not mark the scalp end), samples from 74 women and 40 children were received within 3-4 months of survey completion. This timing was suitable, since 3 cm of hair were used in the analysis, representing approximately 3 months of stress exposure, as hair grows at an average of 1 cm per month (Wennig, 2000). Hair samples were stored at room temperature for up to 12 months prior to analysis.
Hair sample preparation and cortisol extraction: Hair samples were removed from the tape and attached to a Hoffman screw compressor clamp (Science Supply Australia, Vic, AU). Each hair segment was washed thoroughly in isopropanol and dried at room temperature overnight. The most proximal 3 cm of hair to the scalp (where available) was divided into three 1 cm segments and finely chopped into separate glass vials using surgical scissors. One or two 1 cm segments were used where this was all that was available. Samples were weighed (~15-20 mg per sample) and 1 ml of methanol was added to each sample. The vials were sealed and incubated on an orbital shaker at 150 RPM for 16 hours at 52°C. Methanol was transferred to a glass Kimble tube and evaporated under a nitrogen gas stream at 50°C. The remaining residue in the tube was reconstituted in 250 µl of phosphate buffered saline (pH 8.0) and vortexed for 60 seconds. These methods for cortisol extraction from hair samples were based on those used by Manenschijn et al (Manenschijn et al., 2011) and Sauve et al (Sauve et al., 2007).

Hair cortisol analysis:
Cortisol levels were measured using an Enzyme-Linked Immunosorbent Assay for salivary cortisol (Salivary ELISA Cortisol kit©; Alpco Diagnostics, Windham, NH) as per the manufacturer’s protocol. The use of salivary cortisol ELISA to assess cortisol levels in hair has been shown to yield valid and reliable results (Gow et al., 2010; Manenschijn et al., 2011; Russell et al., 2012; Thomson et al., 2010). The intra-assay coefficient of variation was 3.96 % and the inter-assay coefficient of variation was 7.75 %. The inter-assay coefficient of variation was determined by using an internal control sample which was run on each plate. As there was no statistical variation in the cortisol levels among the three 1 cm segments provided by each participant, the analysis was performed on the average cortisol levels across
the three hair segments where available, otherwise one or two segments were used. Three hair segments were available for 93% of women and 83% of children (Table 1). This method for hair cortisol analysis was based on methods used by Manenschijn et al. (Manenschijn et al., 2011) and Sauve et al. (Sauve et al., 2007).

Statistical analyses

Among the 74 women with hair cortisol data, three had extreme outlier values (> 3SD above the mean) for average cortisol level and were excluded, leaving 71 women with hair cortisol data. One of the women excluded was part of a maternal-child pair, therefore data for her child, who also had outlier values, were excluded. Of the remaining 71 women with hair cortisol data, 37 also had hair cortisol data for their child. Three children had extreme outlier (high) values for cortisol and were excluded, leaving 34 maternal-child pairs with hair cortisol data. Participants with missing BMI data were also excluded (n=1 woman, n=4 children); thus data from 70 women and 30 maternal-child pairs were included in the final analysis.

Cortisol data for both women and children were positively skewed, and therefore these variables were log transformed for use in associational analyses. Linear regression models with robust standard errors were used to test bivariate associations between each of the following: 1) women’s hair cortisol level and their BMI, PSS score, their child’s zBMI and hair cortisol level; 2) women’s PSS score and their BMI, their child’s zBMI and hair cortisol level; and 3) children’s hair cortisol level and their zBMI.

Crude and adjusted models for women and children were calculated. For women, education (low, medium, high), smoking status (current, never/former) and the number of children
living in the home (0, ≥ 1) were included in adjusted models, while for children, maternal 
BMI (continuous), maternal education (low, medium, high) and child sex were included. 
Associations with a variety of other potential confounders (age, country of birth, marital 
status, household income, employment status, hair dying) were tested but were not 
statistically significant at p<0.10 and hence these variables were not controlled for in 
analyses.

RESULTS

Characteristics of the women and children included in the analyses are presented in Table 1. 
Women were on average 43 (± 7.2 SD) years of age. The majority of women were born in 
Australia and married. Nearly 43% worked full-time, 41% had a tertiary education and 46% 
reported a household income ≥ $1500 per week. One-third of women were overweight, while 
one-fifth were obese. The mean hair cortisol level for women was 123.7 ng/g (SD=71.2 
ng/g), while PSS scores averaged 5.4 (SD=2.6). Children were 14 years of age on average. 
Twenty percent of children were overweight and 10% were obese. Children’s hair cortisol 
levels averaged 96.6 ng/g (SD=49.6 ng/g).

Scatterplots of women’s hair cortisol levels and their BMI (Figure 1A), women’s PSS scores 
and their BMI (Figure 1B), and maternal and child hair cortisol levels (Figure 1C) are 
presented as Figures. Women's hair cortisol levels were not associated with their BMI or PSS 
scores (Table 2). By contrast, women’s PSS scores were positively associated with their BMI 
in both crude (β=0.71 [95% CI=0.14, 1.28], p=0.015) and adjusted (β=0.65 [95% CI=0.06, 
1.23], p=0.032) models. Within maternal-child pairs, mothers and children’s hair cortisol 
levels were strongly positively associated (crude: β=0.49 [95% CI=0.15, 0.83], p=0.006; 
adjusted: β=0.51 [95% CI=0.17, 0.84], p=0.004). Maternal hair cortisol levels and PSS
scores were unrelated to their child's zBMI (Table 2). Children's hair cortisol levels were not associated with their zBMI or with their mother's PSS score (Table 2).

**DISCUSSION**

Chronic stress represents a bio-behavioural mechanism through which contemporary lifestyles may promote obesogenic behaviours and body fat accumulation. This study examined associations between hair cortisol levels, perceived stress and BMI among women and children living in disadvantaged areas. Findings showed that women’s BMI was positively associated with perceived psychological, but not with a physiological measure of stress, whereas children’s BMI was unrelated to any maternal or child stress measures. There was also no association between women’s perceived stress and their own, or their child’s (in the case of maternal-child pairs) hair cortisol levels. Mother and child hair cortisol levels were, however, strongly positively associated.

The assertion that psychological distress arising from neighbourhood-level disadvantage can increase the risk of obesity derives primarily from cross-sectional studies linking neighbourhood disadvantage with perceived stress (Feldman and Steptoe, 2004; Hill et al., 2005; Ross et al., 2000; Steptoe and Feldman, 2001) or obesity (Ellaway et al., 1997; King et al., 2006; Matheson et al., 2008; Moore and Cunningham, 2012; Robert and Reither, 2004; van Lenthe and Mackenbach, 2002) on the one hand, and connecting perceived stress with obesity on the other (Moore and Cunningham, 2012; Mouchacca et al., 2013; Wardle et al., 2011). Our findings that the perceived psychological stress levels of women residing in disadvantaged neighbourhoods were associated with their BMI are therefore consistent with this body of knowledge. Moreover, as in other studies (Burdette and Hill, 2008; Hill et al., 2005; Matheson et al., 2008), these associations persisted after adjustment for individual-
level measures of SES. Although we were unable to conduct a mediation analysis due to the small sample size, evidence from two other studies suggests that psychological stress may mediate associations between neighbourhood disadvantage and obesity. In the first, psychological distress mediated positive associations between perceived neighbourhood disorder (an index of perceived crime, noise and cleanliness) and obesity risk among a probability sample of 1338 American adults (Burdette and Hill, 2008). In the second, associations among neighbourhood material deprivation, gender and BMI were reduced by 8% when perceived stress and lifestyle and health-related behaviours were accounted for in a representative sample of 64,277 Canadian adults (Matheson et al., 2008). These associations were independent of individual-level measures of SES.

While women’s perceived psychological stress levels were associated with their BMI in the present study, their physiological hair cortisol levels were not. Several studies in other adult populations have similarly found no relationship between hair cortisol levels and BMI (Boesch et al., 2015; Manenschijn et al., 2011; Manenschijn et al., 2013), whereas others have reported small positive associations (Manenschijn et al., 2011; Stalder et al., 2012; Stalder et al., 2013). Manenschijn et al (Manenschijn et al., 2011) reported that although hair cortisol levels were not correlated with BMI in healthy individuals, they were positively correlated with waist circumference. Given that long-term exposure to elevated cortisol levels leads to accumulation of visceral fat (Bjorntorp and Rosmond, 2000), and that BMI is indicative of general and not necessarily abdominal obesity, it is possible that waist circumference, which was not measured in our study, may have proved a more sensitive marker of the exposures we sought to investigate. This conclusion is supported by a recent systematic review which found that abdominal adiposity was associated with increased HPA axis activity, whereas associations with overall adiposity (i.e. BMI) were more mixed.
Previous studies have found significantly higher hair cortisol levels in obese compared to non-obese patients, but no difference in hair cortisol levels between normal and overweight (non-obese) individuals (Manenschijn et al., 2011; Wester et al., 2014). If cortisol levels only become elevated at BMI levels within the obese range, it is plausible that the lack of association between hair cortisol levels and BMI in the current study might alternatively relate to the small number of women who self-reported a BMI in the obese range (n=13). Given the well-known tendency of women to underreport BMI (Merrill and Richardson, 2009), the current findings may be biased towards the null and may have differed had we measured women’s BMI. However, self-reported BMI has been shown to correspond with objective measures in Australian women (Burton et al., 2010). Differences among studies may also partially relate to the use of categorical versus continuous measures of BMI.

The stress response in humans is characterized by multiple physiological and psychological changes. Two of the most prominent responses involve activation of the HPA axis and negative subjective psychological states such as nervousness and tension (Schlotz et al., 2008). Measures of one or both of these response systems can be used to gauge stress levels (Schlotz et al., 2008). Theoretically, physiological and psychological measures of stress should be associated because they presumably represent indicators of the same construct (Schlotz et al., 2008); however in practice this is often not the case (Boesch et al., 2015; Chan...
et al., 2014; Dettenborn et al., 2010; Dowlati et al., 2010; Karlen et al., 2011; Stalder et al., 2012; Van Uum et al., 2008). Similarly in the current study, women’s perceived stress levels were unrelated to their hair cortisol levels. It is possible (Schlotz et al., 2008) that these inconsistencies may reflect different dynamics of the two systems; as whereas subjective psychological responses occur rapidly and change dynamically, physiological HPA axis responses have a slower onset and rate of change (Schlotz et al., 2008). This lag in short-term physiological endocrine behind psychological stress responses is termed a “lack of psychoendocrine covariance,” and has led to speculation that subjective psychological states may mediate associations between stressors and endocrine stress responses (Schlotz et al., 2008). Psychoendocrine cross-correlations may change over time in response to various stressors (Schlotz et al., 2008), however, and therefore it is unclear whether and to what extent findings from short-term studies may be invoked to explain the longer-term stress responses assessed in the current study.

Others have suggested that physiological and psychological stress levels may in fact represent distinct constructs, such that hair cortisol levels reflect a qualitatively different dimension of stress-related exposures than is captured by individuals’ subjective experiences (Stalder et al., 2012). A number of differences between these measures support this notion. For instance, it seems unlikely that a brief four-item questionnaire can capture participants’ experiences of stress across multiple life domains to the same extent as a physiological measure. Alternatively, it might also be said that hair cortisol analysis is limited to providing an indication of overall HPA axis activity over a defined period. In addition, hair cortisol measures are insensitive to brief stressors such as daily hassles that may temporarily elevate PSS scores (Russell et al., 2012). Alternatively, consistent exposure to stress could lead to habituation (Nguyen and Peschard K, 2003), attenuating stress-related appraisals relative to
physiological measures. Notably however, the cortisol response can habituate as well, as initial hypercortisolism has been shown to subside into hypocortisolism with advancing chronicity of stress (Miller et al., 2007). Moreover, emerging evidence indicates that the experience of living in a disadvantaged neighbourhood that one perceives as stressful is associated with hypocortisolism, rather than hypercortisolism (Karb et al., 2012). Future studies comparing the response to stressors across these two systems are essential to clarify the distinctions between psychological and physiological stress responses. Ongoing measurement of both systems appears advisable, given that they may provide complementary information. For instance, perceived psychological measures can augment information gleaned from physiological measures by providing information regarding the source and types of stressors. Diurnal patterns of cortisol activity may also help to explain socioeconomic differentials (Do et al., 2011; Karb et al., 2012; Van Ryzin et al., 2009), and therefore concurrent collection of saliva samples may yield additional insights.

The negative impacts of stress may be particularly damaging to children, as chronic alterations in cortisol secretion could influence development of brain, endocrine and metabolic systems (Hemmingsson, 2014). In light of this plasticity it has been posited that the origins of socioeconomic status-health connections can be traced to stressful conditions experienced by children, particularly those from socioeconomically disadvantaged backgrounds (Finkelstein et al., 2007; Matthews et al., 2010). In addition to environmental influences, genetic heritability or maternal calibration of the child’s HPA axis may also be implicated (Karlen et al., 2013), as studies indicate that high levels of cortisol in hair may be an inherited trait (Fairbanks et al., 2011). In the current study, mother’s and children’s hair cortisol levels were strongly associated, perhaps reflecting shared environmental or genetic influences, or both. We found no evidence that the potentially negative body weight-related
impacts of maternal stressors were transmitted intergenerationally, however, as perceived measures of stress and hair cortisol levels were not associated with measured BMI in children. These findings differ from those of a systematic review that found consistent evidence of associations between reported experiences of stress at the household level and childhood obesity (Gundersen et al., 2011), and subsequent studies finding that psychological stress experienced by parents contributed to obesity risk in children of varying ages and income levels both cross-sectionally (Koch et al., 2008; Parks et al., 2012; Shankardass et al., 2014) and longitudinally (Koch et al., 2008).

Children’s BMI was also unrelated to their own hair cortisol levels. This finding is at odds with evidence indicating that obese children have higher hair cortisol concentrations compared to their non-obese counterparts (Veldhorst et al., 2014). However, our results are consistent with a study which found no relationship between children’s BMI and their salivary cortisol levels (Vanaelst et al., 2014). Given the paucity of studies that have assessed hair cortisol in relation to BMI in children, we can only speculate that the lack of associations observed may reflect the small number of children who were obese in our sample, a lack of measurements of waist circumference to indicate visceral obesity [46], or perhaps the wide variability in their age, as different stressors may influence BMI among younger and older children (Garasky et al., 2009). Children’s body weight is determined by a host of factors originating as far back as the pre-pregnancy period (Barouki et al., 2012; Heindel and Vandenberg, 2015) and it may therefore be the case that other factors were simply stronger determinants of BMI among children in the current sample. Children’s perceived stress levels have been associated with overweight and obesity in other studies (Gundersen et al., 2011; Vanaelst et al., 2014), suggesting that perceived psychological measures of stress may also be more important predictors of weight-related risk among
children as was the case among women in the current study. Nevertheless, we did not measure perceived stress levels among children. Alternatively, the general lack of associations observed between hair cortisol levels and BMI in both women and children in the current study may indicate that the stress levels captured by hair cortisol measures used were not representative of longer-term exposures that may predispose to unhealthy weight gain. Additional studies evaluating associations between perceived psychological and physiological measures of stress and adiposity in larger samples of women and children are essential to clarify these relationships.

**Strengths and limitations**

As this is the first study to examine hair cortisol levels in relation to BMI among disadvantaged women and children, we chose to begin by examining cross-sectional relationships. However, given that body weight is determined over the course of many years, and that stress levels in the past 1-3 months may not reflect longer-term stress levels, longitudinal studies that assess change in BMI in relation to change in stress levels are ultimately needed to understand these relationships. The women in our sample lived in disadvantaged neighbourhoods, however many were relatively advantaged on average according to individual-level measures of SES. It is therefore possible that some women’s greater access to individual-level resources mitigated some of the detrimental impacts of their neighbourhood contexts, potentially masking associations. This might explain why women’s average PSS scores (mean 5.4, SD 2.6) were not markedly elevated compared to those observed in a representative US sample (mean 4.49, SD 2.96) (Cohen and Williamson, 1988). A large number of studies have, however, shown that neighbourhood disadvantage is independently linked to BMI and/or stress after adjusting for individual-level measures of
SES (Burdette and Hill, 2008; Do et al., 2011; Hill et al., 2005; Karb et al., 2012; Matheson et al., 2008).

Because the hypothesized causal pathways were observed simultaneously, it is not possible to determine the direction of causation in relationships between women’s PSS scores and BMI. This study focused on the relationship between stress and BMI, although many other individual, social and environmental factors may also influence BMI. The exclusion of men, the small sample size, and low response rates are further limitations that may limit generalisability of our findings. It is possible that the women and children excluded due to extremely high hair cortisol values may have been using corticosteroid medications, however we are unable to verify this as we did not collect information about medications such as corticosteroid creams. Future studies could compare hair cortisol levels and corresponding associations with BMI between advantaged and disadvantaged groups given that there is no established range of hair cortisol levels (Russell et al., 2012), and a wide range of values have been reported in other studies (Chan et al., 2014; Raul et al., 2004; Sauve et al., 2007; Stalder et al., 2012; Wester et al., 2014). Finally, it is possible that the different time frames captured by our measures (1 month for PSS and 3 months for hair cortisol) could partially account for lack of associations between PSS and hair cortisol levels. However, it appears more likely that it is not the time frame over which stressors were measured that was influential, but that these two measures may capture qualitatively different dimensions of stress.

Despite these limitations this study provides an important platform for future inquiry in this area, and has a number of important strengths. First, we used a physiological measure of chronic stress to complement a perceived psychological measure of stress, integrating both environmental and biological mechanisms to explain weight-related disparities. Second, BMI
was measured in children, although it was based on self-report among women. Finally, although the sample size was small, due to difficulties in finding large numbers of women and children willing to provide hair samples, it was consistent with the sample sizes of similar studies (Chan et al., 2014; Dettenborn et al., 2010; Kalra et al., 2007; Stalder et al., 2012).

CONCLUSIONS

Associations between disadvantage and obesity cannot be fully explained by classic correlates of energy intake and expenditure (Ball et al., 2003; Martikainen and Marmot, 1999; Purslow et al., 2008). It has therefore been hypothesized that the higher levels of stress experienced by individuals living in disadvantaged neighbourhoods may partially explain disparities in body weight between those residing in advantaged and disadvantaged areas. Findings from this study showed that women’s BMI was associated with perceived psychological stress, but not with their physiological hair cortisol levels, whereas children’s BMI was unrelated to any maternal or child stress measures. Physiological and subjective psychological measures of stress may therefore be distinct, where psychological measures may be more important predictors of weight-related risk. Importantly, there was a strong, positive association between maternal and child physiological stress levels. These results provide a platform for future, larger studies that can help to elucidate the pathways linking stress and obesity in women and children living in disadvantaged neighbourhoods and potential intergenerational linkages.

Given the differences observed in associations between a perceived psychological and a physiological measure of stress with BMI, and the fact that hair cortisol analysis provides no information as to the source or types of stressors, future studies should integrate physiological
measures of stress with perceived psychological measures that discriminate between sources and types of stress. Such investigations will provide a more complete perspective of stress-related risk of weight gain, and are better able to inform targets for intervention. Finally if as our findings suggest, stressful conditions perceived by individuals living in disadvantaged neighbourhoods are truly associated with elevated BMI, policies and programs that aim to ameliorate these perceptions should be a high priority.

ACKNOWLEDGMENTS

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DECLARATION OF INTEREST STATEMENT

The authors declare no competing interests.
REFERENCES


Table 1. Characteristics of women and children (n=70 women and n=30 maternal-child pairs)*

<table>
<thead>
<tr>
<th>Women’s characteristics</th>
<th>All women (n=70)</th>
<th>Maternal-child pairs (n=30)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age: mean (SD), range</td>
<td>43.4 (7.2), 23.7-51.1</td>
<td>45.9 (3.9), 37.9-51.1</td>
</tr>
<tr>
<td>Born in Australia: n (%)</td>
<td>65 (92.9)</td>
<td>28 (93.3)</td>
</tr>
<tr>
<td>Marital status: n (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Married/de facto</td>
<td>62 (88.6)</td>
<td>27 (90.0)</td>
</tr>
<tr>
<td>Previously married</td>
<td>4 (5.7)</td>
<td>3 (10.0)</td>
</tr>
<tr>
<td>Never married</td>
<td>4 (5.7)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Education: n (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Did not complete high school</td>
<td>13 (18.6)</td>
<td>3 (10.0)</td>
</tr>
<tr>
<td>Completed high school</td>
<td>28 (40.0)</td>
<td>15 (50.0)</td>
</tr>
<tr>
<td>Tertiary</td>
<td>29 (41.4)</td>
<td>12 (40.0)</td>
</tr>
<tr>
<td>Household income: n (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; $1500 per week</td>
<td>29 (41.4)</td>
<td>11 (36.7)</td>
</tr>
<tr>
<td>≥ $1500 per week</td>
<td>32 (45.7)</td>
<td>15 (50.0)</td>
</tr>
<tr>
<td>Undisclosed</td>
<td>9 (12.9)</td>
<td>4 (13.3)</td>
</tr>
<tr>
<td>≥ 1 child living in the home: n (%)</td>
<td>56 (80.0)</td>
<td>30.0 (100.0)</td>
</tr>
<tr>
<td>Employment status: n (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Full-time</td>
<td>30 (42.9)</td>
<td>9 (30.0)</td>
</tr>
<tr>
<td>Part-time</td>
<td>29 (41.4)</td>
<td>19 (63.3)</td>
</tr>
<tr>
<td>Not in paid employment</td>
<td>11 (15.7)</td>
<td>2 (6.7)</td>
</tr>
<tr>
<td>Smoking status: n (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never</td>
<td>45 (64.3)</td>
<td>16 (53.3)</td>
</tr>
<tr>
<td>Former</td>
<td>21 (30.0)</td>
<td>12 (40.0)</td>
</tr>
<tr>
<td>Current</td>
<td>4 (5.7)</td>
<td>2 (6.7)</td>
</tr>
<tr>
<td>Weight status: n (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Healthy weight</td>
<td>33 (47.1)</td>
<td>13 (43.3)</td>
</tr>
<tr>
<td>Overweight</td>
<td>24 (34.3)</td>
<td>10 (33.3)</td>
</tr>
<tr>
<td>Obese</td>
<td>13 (18.6)</td>
<td>7 (23.3)</td>
</tr>
<tr>
<td>BMI: mean (SD), range</td>
<td>26.2 (6.0), 18.9-46.7</td>
<td>27.4 (7.0), 18.9-46.7</td>
</tr>
<tr>
<td>Hair cortisol level (ng/g): mean (SD),</td>
<td>123.7 (71.2), 42.5-</td>
<td>117.1 (51.3), 45.9-</td>
</tr>
<tr>
<td>range</td>
<td>388.8</td>
<td>227.3</td>
</tr>
<tr>
<td>Number of cortisol measures taken: n (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>2 (2.9)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>2</td>
<td>3 (4.3)</td>
<td>2 (6.7)</td>
</tr>
<tr>
<td>3</td>
<td>65 (92.9)</td>
<td>28 (93.3)</td>
</tr>
<tr>
<td>Hair has been coloured: n (%)</td>
<td>50 (71.4)</td>
<td>20 (66.7)</td>
</tr>
<tr>
<td>PSS score: mean (SD), range</td>
<td>5.4 (2.6), 0-12</td>
<td>4.4 (2.5), 0-9</td>
</tr>
<tr>
<td>Children’s characteristics</td>
<td>n=30</td>
<td></td>
</tr>
<tr>
<td>-----------------------------</td>
<td>------</td>
<td></td>
</tr>
<tr>
<td><strong>Age: mean (SD), range</strong></td>
<td>14.3 (3.9), 10.4-17.8</td>
<td></td>
</tr>
<tr>
<td><strong>Sex: n (%)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>17 (56.7)</td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>13 (43.3)</td>
<td></td>
</tr>
<tr>
<td><strong>zBMI: mean (SD), range</strong></td>
<td>0.3 (1.1), -1.59-2.9</td>
<td></td>
</tr>
<tr>
<td><strong>Weight status: n (%)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Healthy weight</td>
<td>21 (70.0)</td>
<td></td>
</tr>
<tr>
<td>Overweight</td>
<td>6 (20.0)</td>
<td></td>
</tr>
<tr>
<td>Obese</td>
<td>3 (10.0)</td>
<td></td>
</tr>
<tr>
<td><strong>Hair cortisol level (ng/g): mean (SD), range</strong></td>
<td>96.6 (49.6), 41.9-237.7</td>
<td></td>
</tr>
<tr>
<td><strong>Number of cortisol measures taken: n (%)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>3 (10.0)</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>2 (6.7)</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>25 (83.3)</td>
<td></td>
</tr>
<tr>
<td><strong>Hair has been coloured: n (%)</strong></td>
<td>0 (0)</td>
<td></td>
</tr>
</tbody>
</table>

BMI: body mass index; PSS: perceived stress scale; zBMI: age and sex-adjusted body mass index

*A total of 70 women were included in the analysis. Of these women, 30 were also included in the analysis of maternal-child pairs.*
Table 2. Associations between hair cortisol levels, Perceived Stress Scale scores, and body mass index

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Predictor</th>
<th>Crude model</th>
<th>Adjusted model&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>β</td>
<td>95% CI</td>
</tr>
<tr>
<td><strong>Women (n=70)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Women’s BMI</td>
<td>Women’s cortisol</td>
<td>0.20</td>
<td>-2.59, 2.99</td>
</tr>
<tr>
<td>Women’s PSS score</td>
<td>Women’s cortisol</td>
<td>0.30</td>
<td>-0.77, 1.37</td>
</tr>
<tr>
<td>Women’s BMI</td>
<td>Women’s PSS score</td>
<td>0.71</td>
<td>0.14, 1.28</td>
</tr>
<tr>
<td><strong>Maternal-child pairs (n=30)</strong></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Children’s zBMI</td>
<td>Maternal cortisol</td>
<td>-0.07</td>
<td>-0.75, 0.61</td>
</tr>
<tr>
<td>Children’s cortisol</td>
<td>Maternal cortisol</td>
<td>0.49</td>
<td>0.15, 0.83</td>
</tr>
<tr>
<td>Children’s zBMI</td>
<td>Maternal PSS score</td>
<td>0.14</td>
<td>-0.06, 0.34</td>
</tr>
<tr>
<td>Children’s cortisol</td>
<td>Maternal PSS score</td>
<td>0.05</td>
<td>-0.06, 0.07</td>
</tr>
<tr>
<td>Children’s zBMI</td>
<td>Children’s cortisol</td>
<td>0.15</td>
<td>-0.76, 1.06</td>
</tr>
</tbody>
</table>

BMI: body mass index; PSS: Perceived Stress Scale; zBMI: age and sex-adjusted body mass index.

Findings represent effect estimates from linear regression models using robust standard errors; statistically significant results shown in bold.

<sup>a</sup>Models for women controlled for education, smoking status and number of children living in the home, models for children controlled for maternal BMI, maternal education and child sex.
FIGURE CAPTIONS

Figure 1

A Scatterplot of women’s hair cortisol levels and their body mass index (n=70 participants). In adjusted linear regression analyses, women’s hair cortisol levels were not associated with their body mass index ($\beta = 0.13; 95\% \text{ CI } = -2.34, 2.59; p = 0.918$).

B Scatterplot of women’s perceived stress scale scores and their body mass index (n=70 participants). In adjusted linear regression analyses, women’s perceived stress scale scores were positively associated with their body mass index ($\beta = 0.65; 95\% \text{ CI } = 0.06, 1.23; p = 0.032$).

C Scatterplot of maternal hair cortisol levels and their child’s hair cortisol levels (n=30 maternal-child pairs). In adjusted linear regression analyses, mothers’ hair cortisol levels were positively associated with their child’s hair cortisol levels ($\beta = 0.51; 95\% \text{ CI } = 0.17, 0.84; p = 0.004$).