Psychoneuroendocrinology 129 (2021) 105255

Contents lists available at ScienceDirect

Psychoneuroendocrinology

journal homepage: www.elsevier.com/locate/psyneuen

Salivary biomarkers of stress and inflammation in first graders in Côte d'Ivoire: Effects of a probiotic food intervention

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ARTICLE INFO

Keywords:
Cortisol
C-reactive protein
Probiotic
Food supplement
Inflammation
Stress

ABSTRACT

This semi-randomized controlled trial examined the effects of a probiotic food supplement on cortisol and C-reactive protein (CRP) in a sample of 262 four-to seven-year-old children (56% girls) in two economically-disadvantaged schools in an urban setting in Côte d'Ivoire. For one semester, children in one school were randomized to receive a probiotic (N = 79) or placebo (N = 85) fermented dairy food each day they attended school; one child (due to medical reasons) and all children in the other school (N = 98) continued their diets as usual. Children provided two saliva samples at 11:30 on consecutive days at the end of the study. Analyses revealed that the probiotic group had lower cortisol than the placebo or diet-as-usual groups (p = .015); CRP levels were comparable across groups (p = .549). Exploratory analyses suggested that dose and regularity of consumption may impact the biomarkers as well. This study provides the first evidence that a probiotic milk product may lower cortisol in a sample of young, economically-disadvantaged children.

1. Introduction

Stress and inflammation are widely recognized as drivers of poor health. They have been associated with immune dysfunction, cardiovascular disease, depression, burnout, and stroke (e.g., Furman et al., 2019; Hammen, 2005; Iacovides et al., 2003; Kivimäki and Kawachi, 2015; Kivimäki et al., 2013; Lindsberg and Grau, 2003; Mariotti, 2015). Research indicates clear links between the gut microbiota (i.e., the trillions of microorganisms living in the gastrointestinal tract; e.g., De Palma et al., 2015) and both the stress system and systemic inflammation (Misiak et al., 2020). Probiotics, defined as "live microorganisms that, when administered in adequate amounts, confer a health benefit on the host," (Hill et al., 2014) may be a low-cost and accessible means of positively impacting the gut and lowering stress and inflammation. However, the majority of studies on stress, inflammation, and probiotics have been conducted in high income countries in older populations (e.g., Zhang et al., 2020). The goal of the present study was to examine whether regular consumption of a probiotic food, compared to consumption of a placebo or diet as usual, affects biomarkers of stress and inflammation in first-grade children in an urban setting in Abidjan, Côte d'Ivoire.

The study assesses two biomarkers. The first is cortisol, the main effector hormone of the hypothalamic-pituitary-adrenal (HPA) axis and a well-known marker of stress, which is present in the body at rest and elevates in stressful situations. Broadly, cortisol supports health through both immunosuppressant and anti-inflammatory roles in the human

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https://doi.org/10.1016/j.psyneuen.2021.105255
Received 22 December 2020; Received in revised form 26 April 2021; Accepted 2 May 2021
Available online 12 May 2021

body (Bellavance and Rivest, 2014; Wamil and Seckl, 2007). Moreover, given that most cells in the human body contain cortisol receptors, it is thought to play key regulation and support roles for numerous critical systems (e.g., the cardiovascular and metabolic systems), as well as contributing to cellular health and even fetal development (Jones and Gwenin, 2021). When stress is chronic (such as it is in low socioeconomic conditions), cortisol can become permanently elevated, or in cases of extreme stress (e.g., child maltreatment), permanently blunted (Doom and Gunnar, 2013; Young et al., 2020). Higher concentrations of cortisol (measured in hair and saliva) have been associated with cardiovascular risk, depression, and cognitive deficits linked to changes in hippocampal structure (Abell et al., 2016; Kuehl et al., 2015; Lupien et al., 1998). Although the mechanisms underlying many of these links are as yet unclear, studies point to cortisol-induced structural changes in vital organs, such as the hippocampal changes previously mentioned and increased carotid intima-media thickness in the heart (e.g., Tauchmanovà et al., 2002). The second biomarker, C-reactive protein (CRP), is an acute-phase protein produced in the liver. CRP supports the immune system through many mechanisms, including binding of lysophosphatidylcholine expressed on the surface of dead or dying cells and clearing pathogens from the body (Black et al., 2004; Thompson et al., 1999). It is also a marker of systemic inflammation, which, when chronic, is linked with acceleration of neurodegenerative disease, a lessened ability to fight infection (in men), psychiatric disorders, and lower self-rated physical health (e.g., Cunningham et al., 2009; Dinh et al., 2019; Kaspersen et al., 2016; Osimo et al., 2018).

There is evidence that early life experience influences these biomarkers. For example, severe trauma in childhood is associated with elevated cortisol and CRP in adulthood (e.g., van Dammen et al., 2020). Early life stress, including poor nutrition and low socioeconomic status, affects the development of the gut microbiota and plays a critical role in the development of normative stress responses and health (e.g., Gomaa, 2020; de Weerth, 2017). Given this, consumption of foods that positively affect gut health may combat elevated stress and inflammation and be a first step in setting children up for better health later in life.

Probiotics, which are associated with numerous health benefits (e.g., Aponte et al., 2020), have been hypothesized as potential means of reducing both stress and inflammation, but the adult-centered literature is mixed. Regarding cortisol, some randomized controlled trials (RCTs) find probiotic ingestion linked with lower cortisol (e.g., Takada et al., 2016) while others do not (see Zhang et al., 2020 for a meta-analysis). Probiotic use has been associated with lower CRP in unwell populations (e.g., Lowe et al., 2020). However, the only study examining probiotic use and salivary CRP found no association (Harnett et al., 2020), perhaps owing to the fact that serum and salivary CRP are not reliably linked in adult populations (Pay and Shaw, 2019), although in children, they appear to be (e.g., Tvarijonaviciute et al., 2020).

Importantly, no studies have examined these associations early in development when probiotics might have stronger effects, given that critical systems such as the HPA axis are still quite malleable to outside influence (e.g., Tremblay et al., 2020; see Sudo et al., 2004 for evidence in mice). Moreover, the majority of studies examining stress, inflammation, and probiotics have been conducted in WEIRD (white, educated, industrialized, rich, and democratic; Henrich et al., 2010) samples, limiting their generalizability. Populations in low- and middle-income countries (LMICs) have less access to healthcare and, arguably, more risks than WEIRD populations. Children in LMICs often face combined stressors such as food insecurity and social exclusion from economic resources, both of which are associated with higher concentrations of cortisol in hair (Ling et al., 2019; Simmons et al., 2019). Other potential stressors include poverty, malnutrition, environmental toxins, and suboptimal cognitive stimulation (Black et al., 2013; Obradović et al., 2016; Walker et al., 2007). Moreover, limited access to indoor flush sanitation and early bouts of diarrhea, both common in LMICs, are associated with higher CRP in early adulthood (Said-Mohamed et al., 2019). Although no studies in LMICs examine probiotic effects on cortisol or CRP specifically, work in Sub-Saharan Africa and South America shows other health benefits associated with their consumption, such as reduced absorption of environmental toxins, reduced respiratory infections, and shortened duration of acute diarrhea (Bisanz et al., 2014; Basu et al., 2009; Villena et al., 2012). Given this, and the demonstrated safety of probiotic consumption in children of all ages (e.g., van den Nieuwboer et al., 2015), examining probiotic effects on biomarkers of stress and inflammation in children in high-risk settings is a worthy step in the goal of improving health outcomes worldwide.

The current investigation is a semi-randomized controlled design with three arms: probiotic, placebo, and diet-as-usual. It examines whether prolonged probiotic food consumption is associated with salivary cortisol and CRP concentrations in first grade children in Côte d'Ivoire. We hypothesized that participants who consumed the probiotic product over one semester would have lower values of salivary cortisol and CRP than participants who consumed a placebo or no product. We also present sensitivity analyses examining whether results are consistent controlling for body-mass-index and socioeconomic status.

2. Method

2.1. Participants and procedures

Participants were 3- to 8-year-old children recruited from first-grade classes in two closely-situated schools in a low-income district of Abidjan, Côte d'Ivoire. All first-grade children were eligible for inclusion and were recruited via flyers sent home in backpacks, as well as word of mouth and two social events held at the schools. Caregivers provided written informed consent for themselves and their children to take part in the study; children provided verbal assent prior to each instance of data collection. We used a semi-randomized design due to the ethical concern of giving some but not all children in a single school a food supplement. Children in one school (the experimental school) comprised the randomized probiotic and placebo arms; children in the other (the control school) comprised the diet-as-usual arm. In the experimental school all available children were recruited ($N_{probiotic} = 87$, $N_{placebo} = 82$, $N_{DAU} = 1$). In the control school, the first 100 children whose caregivers consented ($N_{DAU} = 100$) were included in the study, resulting in a total sample of 270 children.

Demographics were collected through a caregiver interview that took place either in-person or via telephone. At baseline (T1; prior to randomization) and at outcome (T2; 3–5 months later), children were measured and weighed and provided two saliva samples. They also completed cognitive measures and provided a fecal sample, neither of which are part of current study.

After baseline data collection, children from one school were randomly assigned to receive probiotic (n = 86) or placebo dêguê (n = 83) each day they were in school for 18 weeks (excepting 18 days due to holidays, etc.). Children at the other school (and one at the experimental school who could not have dairy due to health reasons) (N = 101) followed their diets as usual. Medical care was available at no cost to all participants during the study.

The study was approved by the Comité National d'Ethique des Sciences de la Vie et de la Santé in Côte d'Ivoire and the ethics committee of the Social Science Faculty of Radboud University in The Netherlands (ECSW-2018–085R1). The study was registered: AsPredicted #32143. The analyses presented in this paper are secondary to the primary registered question concerning probiotic effects on cognition (separate manuscript currently under review). Not all variables mentioned in the registration were able to be used; analytic techniques were adapted due to missing data.

2.2. Probiotic

Probiotic and placebo dêguê (a West African fermented milk product containing sugar and millet) were produced for this study by a local

dêguê -producing company using standard hygiene practices and 40% less sugar than the company's regular recipe. Probiotic dêguê was fermented using a dried bacterial starter culture with *Lacticaseibacillus rhamnosus* yoba 2012 (previously known as *Lactobacillus rhamnosus* yoba 2012) and *Streptococcus thermophilus* C106 (Kort et al., 2015); and placebo dêguê was made using *S. thermophilus* C106 alone. Labelled sachets containing the ferments were provided to the company by the Yoba-for-Life Foundation. Tests performed in the Netherlands confirmed that the dried starter culture in the sachets contained the expected bacteria. The local dêguê producer was trained on using the Yoba starter culture to produce probiotic fermented dêguê .

Final cell counts were expected to vary between 2 and 9E7 cfu/mL for L. *rhamnosus* yoba 2012 and 0.8–2E9 cfu/mL for *S. thermophilus* C106 (Kort et al., 2015, and see Parker et al., 2018 for the bacterial profile of L. *rhamnosus* yoba 2012 in a similar fermented dish). In-country tests of the product confirmed that it was safe for consumption. Adult consumers reported no difference in the appearance and texture of the two products, but a slightly more sour taste to the probiotic dêguê .

Dêguê was served in 125 mL cups with a snap-on lid. The containers were indistinguishable other than strings of identifying numbers on small labels. Dêguê was delivered to schools in coolers on ice and distributed in classrooms; children were monitored to ensure they only consumed their own dêguê.

2.3. Measures

2.3.1. Covariates

2.3.1.1. Body mass index z-cores. Children's height, weight, age, and gender were used to calculate zBMI using the zscorer package in R, which uses WHO Growth References for school aged children and adults (de Onis, 2007).

2.3.1.1.1. Height and weight. Height and weight were taken on the day children were first asked to provide a T2 fecal sample. Children removed their shoes before both measurements. Height was measured in cm using a stadiometer and weight was measured in kg using a digital scale. Both measures were taken by a single research assistant.

2.3.1.1.2. Age and gender. Caregivers reported their child's gender. Schools and caregivers provided birthdays. When reporters only provided a year, the child's age was calculated as if their birthday were July 1 of the year indicated ($N = 34$). When only a month and year was given, the child's age was calculated as if their birthday were the first day of the month and year provided ($N = 3$). If reporters only provided a month and day or only a month, the birthday was considered to be missing. One birthday was discarded as it seemed implausible (i.e., parents reported an age of 15 years, but the child was clearly much younger). Ages are in days on the day each child provided a T2 stool sample (within two weeks of providing saliva samples).

2.3.1.2. Socioeconomic status (SES). Caregivers endorsed which of 20 household items (e.g., a stove; created in consultation with local researchers) they owned, a common procedure for estimating SES in low-income contexts (e.g., Fotso and Kuate-Defo, 2005). Scores are the number of items endorsed; higher scores reflect higher SES.

2.3.2. Product consumption

2.3.2.1. Dose: total milliliters consumed. Researchers recorded when children were present and consumed 125 mL of dêguê . Unfinished servings were weighed (in grams); the amount remaining was subtracted from 125 to approximate how much was consumed. Scores reflect the sum of the total milliliters of dêguê consumed from the first day of the intervention until prior to T2 testing (within two weeks of saliva collection).

2.3.2.2. Regularity of consumption. Children were defined as "regular consumers" when they consumed some dêguê on at least two days in every week it was offered, not including one, prior to testing. This variable was scored as regular = 1 and non-regular = 0.

2.3.3. Salivary biomarkers

At T1 and T2, children provided two saliva samples on consecutive days by spitting into a 25 mL tube labeled with a line, resulting in four samples for each child. Saliva collection, which was done by class, always occurred around 11:30 am, a time when children would have been sitting in class for the last hour without food or strenuous physical activity. Children were monitored to ensure they only spat in their own tube. Immediately after collection, samples were transferred to 1.5 mL tubes and transported on ice to a local research facility where they were kept at −20 degrees Celsius. Due to a technical conservation error at the lab, 57% of T1 samples, all from the experimental school, were rendered unusable and were discarded. At study completion, the remaining saliva samples were packed in dry ice and sent to the Netherlands for processing. Saliva was processed at the Central Diagnostic Laboratory's Specieel Laboratorium at Utrecht University Medical Center. Only T2 values are used for the current analyses.

2.3.3.1. Cortisol. Cortisol in saliva was measured without extraction using an in house competitive radio-immunoassay employing a polyclonal anticortisol-antibody (K7348). [1,2–3 H(N)]-Hydrocortisone (PerkinElmer NET396250UC) was used as a tracer. The lower limit of detection was 1.0 nmol/l and inter-assay variation was < 8% at 2.6–31 nmol/l (n = 45). Intra-assay variation was < 4% (n = 10).

2.3.3.2. CRP. CRP was measured using the Salivary C-Reactive Protein ELISA Generation II (1–2102, Salimetrics, USA). The lower limit of detection was 0.2 pmol/L. Inter-assay variation was < 5% at 0.5 – 5.2 pmol/L (n = 30). Intra-assay variation was < 1,5% at 0.3 – 2.6 pmol/L (n = 5).

2.4. Statistical analyses

Cortisol and CRP concentrations were averaged over the two T2 sampling days and used for analyses. Experimental group effects on cortisol and CRP values were examined with ANOVAs using complete case analysis without covariates. Then, ANCOVAs with SES and zBMI scores chosen *a priori* as covariates were run as sensitivity analyses. We had no missing values for cortisol or CRP, but some for SES and zBMI. In the probiotic and placebo groups, exploratory analyses examined potential effects of probiotic dose and regularity of consumption using regression analyses including group, mL consumed / regularity of consumption, and the group* mL consumed / regularity of consumption interaction. These analyses were also run using complete case analysis, first without covariates, and then with.

3. Results

3.1. Preliminary analyses

Only children who provided saliva samples at T2 were included in the current analyses (N = 263; $N_{probiotic} = 85$, $N_{placebo} = 79$, $N_{DAU} = 98$; Fig. 1), due to loss of T1 samples by the lab. Analyses examining demographic differences between the experimental and control schools revealed that control school caregivers had higher SES than experimental school caregivers (Table 1).

Probiotic or placebo dêguê was provided to experimental school participants every weekday from January 14, 2019 to May 15, 2019 with the exception of 20 days due to production issues, national holidays, and school testing days. Children had between 53 and 68 opportunities to consume dêguê prior to outcome data collection. Of these

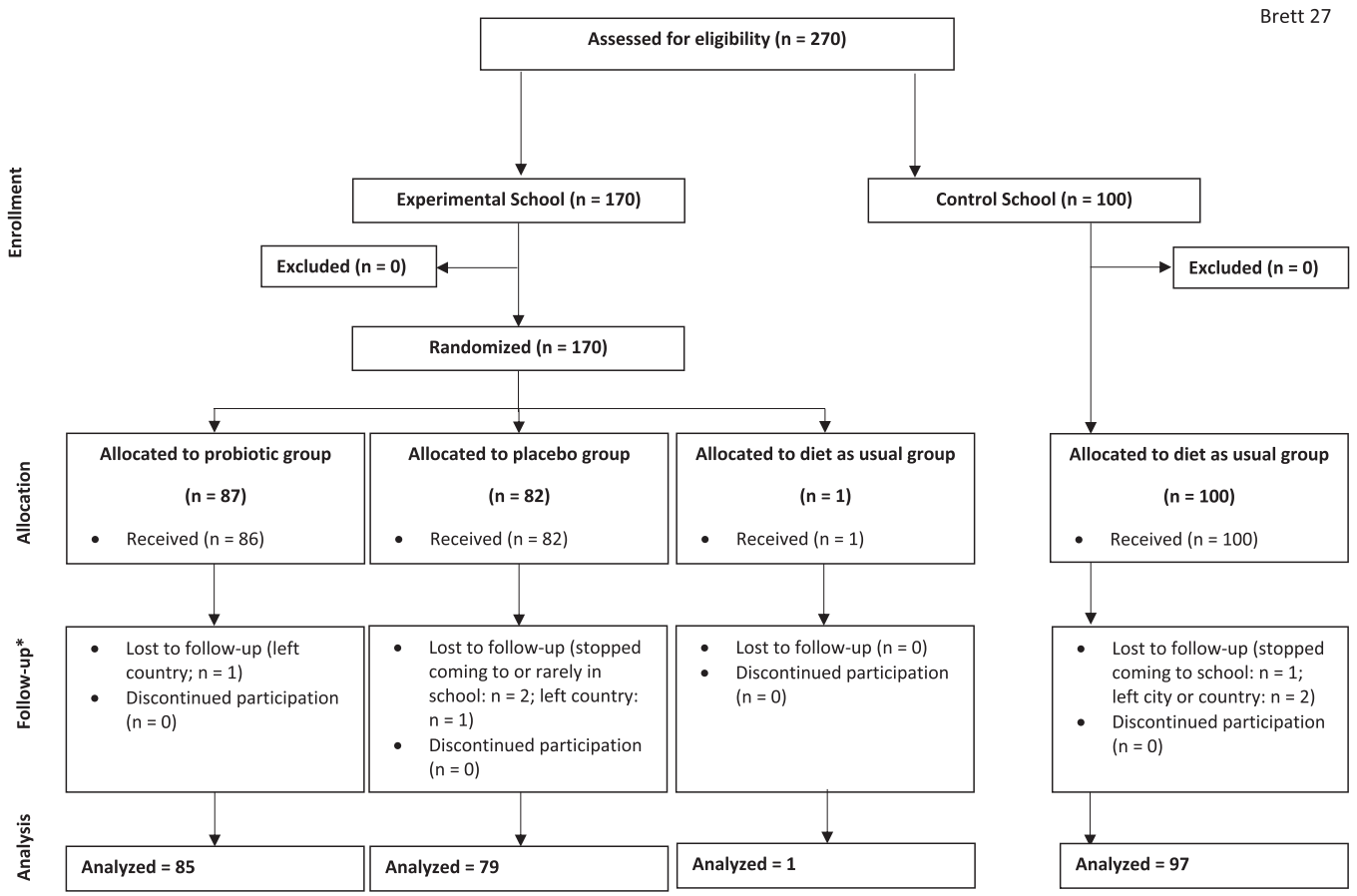

Fig. 1. Participant consort flow chart.

Table 1
Sample demographics and school comparison.

	Sample	By Group			
		Experimental School		Control School	
Variable (range)	(n = 262) *M (SD) N*	Probiotic (n = 85) *M (SD) N*	Placebo (n = 79) *M (SD) N*	Diet as Usual (n = 98) *M (SD) N*	*Test statistic*[a]
Gender (% girls)	56.11	62.35	48.10	57.14	.17[b]
Age in years	5.97 (0.74)	6.08 (0.88)	5.92 (0.73)	5.94 (0.62)	.56[c]
(4.01 – 8.86)	226	68	69	89	
SES	9.47 (2.48)	8.64 (2.37)	9.12 (2.39)	10.40 (2.34)	**-4.69***[c]
(3 – 17)	245	76	74	95	
T2 Height in cm	115.38 (6.10)	116.40 (6.90)	114.68 (6.14)	115.07 (5.22)	.86[c]
(99.90 – 137.20)	253	82	76	95	
T2 Weight in kg	19.46 (2.92)	20.06 (3.50)	19.07 (2.84)	19.24 (2.32)	1.29[c]
(13.1 – 34.0)	259	84	77	98	
T2 BMI z-score	-0.60 (0.83)	-0.52 (0.89)	-0.66 (0.86)	-0.62 (0.78)	.46[c]
(−2.73 to 3.13)	223	68	67	88	
mL degue consumed	4144.96 (1086.22)	4121.84 (990.49)	4169.85 (1186.54)		
(970 – 6344)	164	85	79		
Consistent consumers (% consistent)	26.22%	22.35%	30.37%		
T2 Cortisol[d]	8.31 (2.59)	7.64 (2.53)	8.58 (2.50)	8.66 (2.62)	
(1.00 – 18.63)	262	85	79	98	
T2 C-reactive Protein[d]	3.16 (6.76)	3.27 (6.88)	2.21 (4.52)	3.84 (8.02)	
(.19 – 43.01)	262	85	79	98	

Note. M = Mean. SD = Standard Deviation. SES = socioeconomic status. mL = milliliters.
[a] test statistics are for differences between the experimental school and control school.
[b] $\chi 2$ tests for categorical data.
[c] Independent samples *t*-tests for normally distributed data.
[d] Data are means over two sampling days (11.30hs), non-transformed, and winsorized.
* $p < .001$

opportunities, 33 occurred during a nationwide teacher strike in which schools were closed but the research team was present. During the strike, the team attempted daily contact to encourage caregivers to bring children to school for dêguê . The mean number of days all enrolled children consumed dêguê over the whole study period was 33.65 (SD = 10.97; $M_{probiotic}$ = 33.64, *SD* = 10.31; $M_{placebo}$ = 33.66, *SD* = 11.69). Experimental groups consumed similar quantities of dêguê ($t = -0.03$; $p = .778$) and had a statistically equivalent number of "regular" consumers ($\chi 2 = 1.36$, $p = .243$; Table 1). Children appeared to enjoy the product: more than 95% of cups given were finished. No adverse health effects of the product were reported. Approximately 12% of the sample sought medical care for minor illnesses or injuries.

Height and weight values were checked for outliers and inconsistencies. In particular, we examined cases where values indicated that children had grown shorter (N = 5), had grown more than 8 cm (N = 4), or whose weight changed more than 3 kg (N = 5) from T1 to T2. In all cases, if only one value was biologically plausible it was kept and the other was marked as missing. In cases where both values were plausible or implausible, both were marked as missing.

T2 Cortisol and CRP (N = 524) values were checked for outliers. All values were biologically possible, so extreme values (N = 19) were winsorized (i.e., replaced with a value 3 standard deviations above the mean; Tukey, 1977). Cortisol values below 1 were set at.99 (N = 4 in all T2 samples) and CRP values below.2 were set at.19 (N = 137 in all T2 samples). Cortisol values, which were normally distributed, were averaged across the two T2 samples ($r = 0.38$, $p = .000$) to create a T2 cortisol score. The distribution of CRP values was highly skewed (> 3) for both T2 samples; log transformation resulted in acceptable skew values (< 1.1). Log transformed CRP values were averaged across the two T2 samples ($r = 0.79$, $p = .000$) to create a T2 CRP score.

Sample characteristics as well as mean T2 cortisol and (untransformed) CRP values are presented in Table 1.

3.2. Main analyses

3.2.1. Cortisol

A one-way ANOVA revealed a group effect on T2 salivary cortisol concentrations (F [2259] = 4.25; $p = .015$). Fisher's Least Significant Difference (LSD) test revealed that children in the probiotic group had lower salivary cortisol at T2 than either the placebo or diet as usual groups (Table 1; Fig. 2). The difference became marginally significant with the inclusion of covariates, perhaps due to loss of sample size (F [2203] = 3.03; $p = .051$).

3.2.2. CRP

There were no group effects on T2 log CRP values, with (F [2259] =0.60; $p = .549$) or without (F [2203] =0.59; $p = .553$) covariates (Table 1).

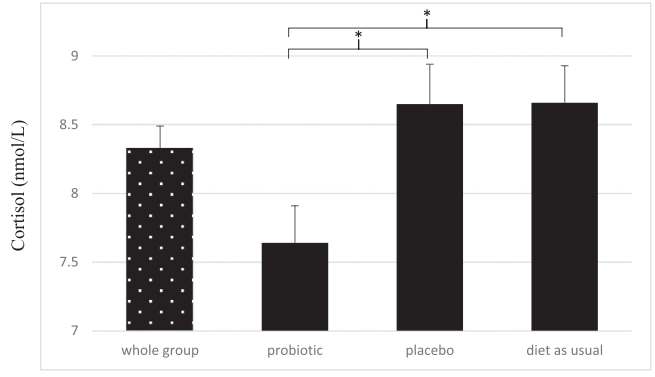

Fig. 2. Cortisol means by group. *Note.* Error bars are standard errors of the mean. Significant differences ($p < .05$) marked with a star.

3.3. Exploratory analyses within experimental groups

3.3.1. Cortisol

In a dose response analysis (i.e. with total milliliters consumed), group but not dose (nor the interaction of the two) predicted T2 cortisol values; placebo group membership predicted higher cortisol (Table 2). Analyses examining regularity of consumption revealed main effects of regularity (with more regular consumption predicting higher cortisol) and group (with placebo group membership predicting higher cortisol), but the interaction of regularity and group was not significant (Table 2). For both analyses, inclusion of covariates rendered the models non-significant.

3.3.2. CRP

In dose response analyses, there was a significant interaction between group and dose. Simple slopes analyses revealed that greater consumption of the placebo product was associated with lower T2 CRP values (Fig. 3). The model with covariates was not significant. No models predicting T2 CRP with regularity of consumption reached significance (Table 2).

4. Discussion

This is the first study to show a link between probiotic ingestion and reduced cortisol in an economically-disadvantaged population of

Table 2
Exploratory Regression Analyses Predicting Cortisol and CRP from mL Dêguê Consumed and Regularity of Consumption Presented Without and With Covariates.

	mL of dêguê consumed				Regularity of consumption		
	ß	*b*	*p*		*ß*	*b*	*p*
T2 Cortisol	***F* (3, 160) = 2.83, *p* = .040**				***F* (3, 160) = 4.60, *p* = .004**		
Constant		8.10	**.000**	Constant		8.12	**.000**
Group	.18	.92	**.021**	Group	.17	.85	**.030**
mL consumed	.13	.000	.095	Regularity	.19	1.11	**.013**
Gp*mLc	-0.03	.000	.685	Gp*R	-0.12	-1.38	.120
R^2	.05			R^2	.08		
T2 Cortisol	***F* (5, 117) = 1.15, *p* = .337**				***F* (5, 117) = 1.64, *p* = .154**		
Constant		8.00	.000	Constant		8.02	.000
SES	.02	.02	.847	SES	.02	.02	.860
BMIz	.07	.21	.445	BMIz	.07	.20	.457
Group	.18	.93	.053	Group	.18	.91	.056
mL consumed	.11	.000	.262	Regularity	.15	.87	.100
Gp*mLc	-0.04	.000	.675	Gp*R	-0.11	-1.21	.248
R^2	.05			R^2	.06		
T2 CRP	***F* (3, 160) = 2.67, *p* = .050**				F (3, 160) = 1.44, $p = .234$		
Constant		-0.10	**.029**	Constant		-0.09	.041
Group	-.04	-0.05	.591	Group	-.04	-0.04	.621
mL consumed	-0.09	.000	.270	Regularity	-0.05	-0.06	.586
Gp*mLc	-0.18	.000	**.023**	Gp*R	-0.15	-0.37	.065
R^2	.05			R^2	.03		
T2 CRP	***F* (5, 117) = 1.62, *p* = .1260**				***F* (5, 117) = 0.81, *p* = .542**		
Constant		-0.09	.666	Constant		-0.11	.598
SES	.01	.003	.867	SES	.02	.01	.831
BMIz	.12	.07	.200	BMIz	.10	.06	.293
Group	-.01	-0.01	.946	Group	-.02	-0.02	.860
mL consumed	-0.08	.000	.381	Regularity	-0.06	-0.07	.549
Gp*mLc	-0.21	.000	.030	Gp*R	-0.14	-0.34	.139
R^2	.07			R^2	.03		

Note. *ß* = standardized regression coefficient; *b* = regression coefficient; R^2 = total variance explained by the model. #s = numbers. mL = milliliters. CRP = C-Reactive Protein. Gp*mLc = group by milliliters consumed interaction. Gp*R = group by regularity classification interaction. BMIz = body mass index z-scores. Significant models and significant effects within them are bolded.

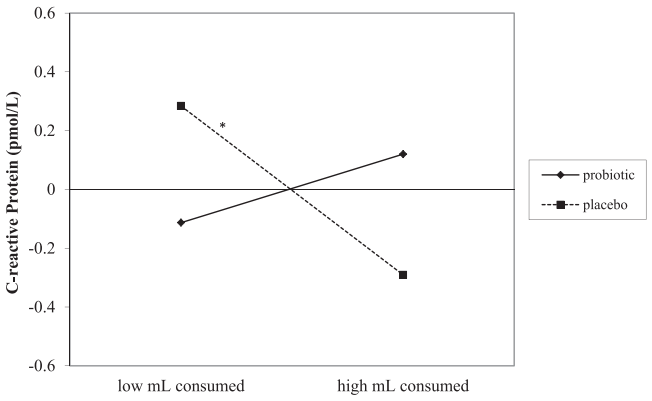

Fig. 3. Interaction of Group and Milliliters Consumed (Dose) on log transformed T2CRP values. Note: * placebo slope is significant.

children from a LMIC. One possible underlying mechanism for reducing stress could be via the ingested bacteria enhancing the gut barrier function. This would then prevent inflammatory mediators from passing into the bloodstream and crossing the blood brain barrier where they would stimulate HPA axis reactivity and increase cortisol production (Misiak et al., 2020). This is supported, albeit by an animal study, in a study in which a *Lactobacillus farciminis* treatment suppressed such stress-induced hyperpermeability, thereby attenuating HPA reactivity to stressful events (Ait-Belgnaoui et al., 2012). It is possible that a simple boost to gut health through semi-regular consumption of a probiotic milk product induced similar changes in the current sample. Future investigations could perform metabolomic analyses to identify compounds in the blood that originated from the gut microbiota as well as immune markers of inflammation. Discerning mechanisms of probiotic effects on stress will advance not just our understanding of the gut-brain link, but also of ways to positively capitalize on it for optimal human health.

The CRP concentrations at the end of the study were comparable between groups (Table 1). This null finding is consistent with the only other study examining probiotics and salivary CRP (Harnett et al., 2020). It is possible that probiotic ingestion is unrelated to salivary CRP values or that semi-regular consumption was insufficient to reduce systemic inflammation. It is also possible that the CRP values in the current investigation were influenced by oral health, a known phenomenon that may be more common in countries with lower access to dentistry (Szabo and Slavish, 2021). Of note, many children had very low levels of CRP suggesting that this may not have been the case and in fact, that participants may have been relatively healthy at the outset. A study of known malnourished or ill children would reveal if the probiotic supplemented food affects CRP.

Interestingly, no dose response effect was found on cortisol. It is possible that any amount of L. *rhamnosus* above a certain threshold can attenuate cortisol levels. Among the children in the experimental groups, those who consumed either product regularly during the study had the highest cortisol. It is possible that children from more food insecure or stressful households were sent out of the home more often and had more opportunities to come to school for a food supplement, even during the teacher strike. Note also that the placebo group had more regular consumers than the probiotic, which could account in part for this seemingly contradictory finding, given that they had higher cortisol.

There was a significant interaction between dose and group predicting CRP: milliliters of dêguê consumed was associated with lower CRP in the placebo group only (Fig. 3). It is possible that the *S. thermophilus* used to ferment the placebo product has undiscovered anti-inflammatory properties. However, this organism was also present in the probiotic dêguê making it unlikely that this explains the difference. More likely, it was an effect of chance owing to the high number of children with low levels of salivary CRP and the model becoming insignificant upon addition of covariates. Replication will be critical to confirm the present findings, as will continued research to determine dosage and timing effects in diverse populations.

The current investigation had many strengths. The study was semi-randomized, had three arms, and comprised a relatively large, non-WEIRD population of children. The probiotic and placebo dêguê were well liked and not associated with any adverse effects. The study had high retention, with only six children lost to follow-up. We took saliva samples at the end of the morning, a moment we knew the children would have been inactive for the hour prior, and at the same time on both sampling days. Having two assessments on consecutive days helped to account for normal daily fluctuations. The study limitations included being unable to deliver the probiotic and placebo dêguê as consistently as intended due to the teacher strike, and the loss of baseline samples preventing examinations across time and baseline group comparison. However, groups did not differ on any baseline covariates except SES and controlling for SES in the analyses did not greatly impact the cortisol results. In addition, we were unable to assess children's subjective stress and health levels due to their young age and difficulties with self-reporting discovered during pilot testing. Given that cortisol is linked with many facets of functioning, including but not limited to stress, such information would help to disentangle mechanisms of effect and should be included in future investigations. Limitations are to be expected when working in challenging LMIC settings. Nonetheless, future studies should endeavor to deliver probiotic products consistently in order to fully elucidate their effects, should compare outcome values to a baseline where possible, and should measure subjective experiences of stress and health where possible.

4.1. Conclusions

Overall, this study showed that L. *rhamnosus* Yoba-2012 might lower cortisol in young, economically-disadvantaged children. This, combined with the finding that the probiotic dêguê was well received and readily consumed, shows the potential to harness the benefits of probiotics and disseminate them in communities at risk for early life stress. Given their affordability and other well researched health benefits, probiotics may lead the way to a healthier population, particularly in LMICs. Future studies should investigate the full scope of probiotics and other fermented foods on early biomarkers of health with the aim of setting children on a positive health trajectory early in life.

Funding

This work was supported by a Jacobs Foundation Advanced Research Fellowship and a personal VICI grant from the Netherlands Organization for Scientific Research (016. Vici. 185.038) to C. de Weerth.

CRediT authorship contribution statement

Bonnie E. Brett: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Writing - original draft, Writing - review & editing, Visualization; **Bruno Koko**: Investigation, Methodology, Project administration, Resources, Writing - review & editing. **Habib O. Y. Doumbia**: Investigation, Methodology, Project administration, Resources, Writing - review & editing. **Frédéric Kouadio Koffi**: Investigation, Methodology, Writing - review & editing. **Savorgnan E. Assa**: Investigation, Methodology, Writing - review & editing. **Kollet Y. A. S. Zahé**: Investigation, Methodology, Writing - review & editing. **Hortense Faye-Ketté**: Resources, Methodology, Supervision, Writing - review & editing. **Sérafin Kati-Coulibaly**: Resources, Methodology, Supervision, Writing - review & editing. **Remco Kort**: Conceptualization, Methodology, Resources, Writing - review & editing. **Wilbert Sybesma**: Conceptualization, Methodology, Resources, Writing - review & editing. **Gregor Reid**: Conceptualization, Methodology, Writing - review & editing. **Carolina de Weerth**:

Conceptualization, Data curation, Funding acquisition, Methodology, Project administration, Resources, Supervision, Writing - original draft, Writing - review & editing.

Declaration of Competing Interest

WS and RK developed the starter culture sachets and founded the Yoba for Life Foundation, a non-profit networking organization aiming to improve health and wealth of people in developing countries by facilitating local production of probiotic fermented food. WS and RK gain no financial benefits from their Yoba for Life activities.

Acknowledgements

We thank the many contributors who helped make this study happen. We are especially thankful for our local partners, too numerous to name but including: the school directors and teachers who helped us facilitate saliva collection, the government officials who helped us find the families and schools to participate in this study, our partners from the Jacobs Foundation at TRECC (Transforming Education in Cocoa Communities) who helped us navigate the local landscape and gave us a home from which to operate, our esteemed research partners who were integral to keeping the study running even in the face of political turmoil, and the families and children who were kind enough to be a part of the study. We also thank Dr. Nieke Westerik for the yoghurt-production training she provided.

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