

Pre-Analysis Plan: Maternal Stress and Food Preferences

Michèle Belot, Jonathan James, Martina Vecchi and Nicolai Vitt*

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1) Proposed Study

The spread of obesity in developed nations over the past decades has disproportionately affected lower socioeconomic groups as they face a larger risk of overweight and obesity (McLaren, 2007). Among the factors suggested to cause this socioeconomic gradient in obesity are higher levels of stress and a lack of tools to cope with stressful situations among low socioeconomic groups (Moore and Cunningham, 2012).

Descriptive evidence has shown (some) individuals exposed to chronic stressors to have a less balanced diet and an increased calorie intake (Torres and Nowson, 2007). Lab experiments have shown acute stress to shift individuals' food choices towards a less healthy diet with higher consumption of sugars and fat and a higher total calorie intake (Rutters et al 2009, Zellner et al 2006).

Studies by Epel et al (2001) and Newman et al (2007) suggest cortisol might play a vital role in the link between stress and dietary behaviours, their findings show significant changes in food choices only among subjects with a strong cortisol response to stress. One potential cause of this variation in cortisol reactivity and in dietary responses to stress is the abilities to cope with stress (Newman et al, 2007).

The aim of the study proposed here is to further examine the relationship between chronic and acute stress, coping abilities and dietary choices. In particular, we plan to focus on the following two questions:

Firstly, we plan to study the effects of acute stress and coping abilities on food choices both in the context of immediate consumption (a “snack choice”) and planned consumption (a “food shopping choice”). Previous laboratory experiments have focused on immediate consumption choices. Food choices made in supermarkets and shops, however, are not aimed at immediate consumption and hence are based on different decision processes.

We propose two channels through which stress might affect food choices: (1) by affecting individuals' preferences and (2) by affecting their ability to make sound decisions. Hormonal responses to stress have been frequently cited to cause cravings for energy-dense “comfort foods” and hence a (temporary) change in food preferences (e.g. Adam and Epel, 2007). Both acute and chronic stress stimulate the release of cortisol (in humans) or of other glucocorticoids (in animals) in the

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hypothalamic-pituitary-adrenal (HPA) axis which in turn have been shown to affect food intake of rats (Zakrzewska et al, 1999; Dallman et al, 2004) and humans (Tataranni et al, 1996; George et al, 2010) when administered exogenously.

On the other hand, stress can be a drain on resources such as mental energy and time which are necessary to make sound decisions (Allen and Armstrong, 2006). Experimental evidence shows cognitive overload to impair self-control and lead to less healthy food choices (Shiv and Fedorikhin, 1999). Stress has furthermore been found to temporarily alter time preferences and risk attitudes (Delaney et al, 2014; Kandasamy et al, 2014) thereby affecting the ability to make decisions which optimize long-term utility.

The relative importance of these two proposed channels is expected to differ between food shopping and immediate consumption choices. Stress-induced food cravings (1st channel) are likely to play a somewhat smaller role for shopping than for immediate consumption choices. As shopping choices require planning of future consumption and often involve larger choice sets, impaired decision making (2nd channel) would be expected to affect these choices more than the less complex consumption choices.

The second question we plan to study concerns the effects of mothers' chronic stress during pregnancy and their ability to cope with this stress on the food preferences of their children. Food preferences and food behaviour patterns are formed to a certain extent during pregnancy and to a larger extent during early childhood (Birch, 1999). Current research in the medical sciences highlights the role of maternal stress during pregnancy and the resulting hormonal processes in determining children's obesity risk in later life via so-called "fetal programming" (Entringer, 2013; Entringer and Wadhwa, 2013). In addition to this hormonal process, stress is likely to affect mother's dietary choices during pregnancy and early childhood which in turn are primary influences on the formation of children's food preferences (Nehring et al, 2015).

Our experimental design will furthermore allow us to explore additional questions such as the role of time preferences and risk attitudes for the effect of stress on dietary choices.

To study these research questions we propose a two group experimental design with a pre-assignment of experimental conditions at the lab session level ensuring balance in terms of day of the week and time of day. Sessions will be pre-assigned to the stress treatment or to the control group. In the stress treatment, subjects will be asked to complete a 10 minute block of short incentivised decision tasks. While the tasks will be completed individually, incentives are based on the joint performance of randomly assigned "social groups" of 2 subjects to elicit social stress. Incorrect answers and incomplete tasks are penalized. Time pressure is induced by the tight overall time limit as well as by time penalties and time limits per task. Short incentivised knowledge questions will randomly appear on screen over the course of the task block to induce additional stress through distractions. Our stress protocol is designed to mimic stressors often experienced by low-socioeconomic mothers: making decisions with consequences for others (e.g. for the family) subject to financial and time constraints as well as distractions (e.g. by children requiring attention). The effectiveness of our stress protocol will be tested using a survey question on the perceived stressfulness of the task as well as the change in heart rate and salivary cortisol in response to the task.

In the control group, participants are asked to read several short texts on a variety of topics and answer simple non-incentivised questions about these texts.

Following the stress or control task, subjects will be asked to use a fixed budget to purchase food items in a "virtual supermarket", a computer-based tool similar to online supermarkets. A variety of healthy and unhealthy food and drink items is available to choose from with prices matching market prices at a local supermarket. Nutritional content of the chosen basket of food and drink items will be used to determine the impact of acute stress on the healthiness of food shopping choices.

Following the before mentioned food shopping choice, subjects will be given a five minute break and then asked to complete a questionnaire on demographics and other control variables. A choice of snacks involving high- and low-calorie snack foods will be placed in bowls on each subject's desk for consumption during the break and during the completion of the questionnaire. The amount of each snack type consumed by each subject will be recorded by weighing the snack bowls before and after the snacks are available. The resulting quantities will be used to determine the impact of acute stress on the healthiness of snacking choices.

In addition to the randomly assigned acute stress treatments, our study will consider several explanatory variables which are not randomly assigned. Chronic stress during pregnancy will be measured using a questionnaire on specific stressors and their perceived stressfulness. Coping abilities of mothers will be measured using questions about coping style and in the stress treatment group using the HR and cortisol response to the stress task.

2) Experimental Design

The sessions for this experiment will take place between 18 June and 26 June 2018 in computer laboratories at the University of Florence, Italy. Sessions will last approximately two hours and will start at 12:00 (noon) or at 3:30 pm. We employ a between subject design, with subjects only attending one session. The session slots have been pre-assigned to the two experimental conditions.

We plan to conduct a second study with a similar design in the United Kingdom in the autumn of 2018, for which we aim to recruit a sample of between 100 and 200 mothers. The experimental design will be adjusted to the UK context, in particular the questions relating to diet, and the products offered in the virtual supermarket and in the snack choice.

a) Sample & Recruitment Procedure

We aim to recruit up to 100 low-income mothers living in Florence, Italy. The specific eligibility criteria for participation in the study are:

- 1) Aged between 18 and 45
- 2) Fluent in Italian
- 3) Mother whose youngest child is aged between 2 and 12 years old
- 4) Live in a household with at least one other adult
- 5) Net annual household income below 33 000 €
- 6) Does not hold a university degree and is not currently enrolled at university
- 7) Has not been pregnant in the past 6 months
- 8) Has no allergies or intolerances to foods used for the snack consumption choice
- 9) Does not have medical conditions which can affect diet

Additional criteria which are not mandatory for participation, but which were checked during registration, are:

- 10) Has not been nursing in the past 6 months
- 11) Does not currently receive regular medical treatment
- 12) Does not take oral contraceptives
- 13) Does not have medical conditions which can affect cortisol
- 14) Has not experienced onset of menopause
- 15) Has no allergies (to food or non-food substances)

Criteria 10) to 15) were applied as these factors can affect cortisol levels and cortisol reactivity.

Participants will be recruited using multiple channels. An external recruitment agency will distribute leaflets in low-income neighbourhoods of Florence. Further recruitment channels include advertisement on social media and promotion of the study to non-academic university employees.

Those interested in participation are invited to complete an online screening questionnaire or contact the experiment team by telephone. Eligible mothers will then be invited to one of the experimental sessions. A second adult living in the same household will be asked to complete a short paper questionnaire at home, which the mothers then bring to the experimental session.

b) Randomization

We plan to conduct up to 14 experiment sessions with up to 18 participants per session. The 14 sessions will be spread over a period of 9 days.

The experiment will follow an experimental design with 2 experimental conditions:

- 1) Stress Task
- 2) Control Task

These experimental conditions will be pre-assigned at the session level. This pre-assignment of sessions to experimental conditions ensures balance in terms of day of the week and time of day.

When signing up for participation in the experiment, participants are asked to indicate their preferred session slots, but will not be informed in advance of the treatments associated with each time slot. If participants indicate availability for multiple slots, they are assigned to one of the slots solely based on scheduling concerns.

Participants will receive an information leaflet, a consent form and a short paper questionnaire for the second adult in the household via post. They are asked to bring the signed consent form and the completed paper questionnaire on the day of their session.

c) Procedure

Upon arrival at our lab facilities, participants' body weight and body height is measured without shoes and heavy clothing. Throughout the experimental session, participants are asked to wear an armband monitoring their heart rate using an optical sensor. At the beginning of the experimental session, participants are asked to provide a first saliva sample.

Following this, participants are asked to complete a 10 minute task. The nature of the task depends on the session's randomly assigned experimental condition:

- In condition 1) (detailed above), i.e. the stress treatment group, participants are asked to complete an incentivised task aimed at inducing mild stress.
- In condition 2), i.e. the control group, participants are asked to complete a task of similar nature but with no stress inducing features.

Detailed descriptions of these tasks can be found below.

Following the first task, participants are asked to complete a "food shopping" task. They are given a fixed budget of 25€ to purchase grocery items in a "virtual supermarket", a computer-based tool similar to online supermarkets. Details of this food shopping choice are outlined below.

After the "food shopping" task, participants are asked to provide a second saliva sample (approx. 25-30 mins after start of stress / control task) and then given a five minute break. After the break, participants are asked to complete a questionnaire on demographics, family characteristics and behaviours which might impact cortisol levels. During the break and the time given to complete the first questionnaire, participants are given permission to consume the snacks provided on their desks: a bowl of high-calorie and a bowl of low-calorie snacks (not labelled as such or in any other way). After completion of the first questionnaire, the bowls of snacks are collected.

Participants are then asked to complete a second questionnaire. The questionnaire features questions about food consumption and food preferences of the participant and their youngest child as well as the participant's food consumption during pregnancy. The questionnaire furthermore includes questions about the stressfulness of the stress/control task, chronic stress, participants' coping behaviours when

dealing with stress and about potentially stressful events during the last 3 months as well as during the pregnancy.

At the end of the experimental session, a final saliva sample is collected (approx. 80-90 mins after start of stress / control task).

Before receiving their payment, participants are debriefed: It is explained that the snacks provided differed in calorie content. Contact details are provided in case of questions, complaints or other problems.

d) Stress Treatment

In the stress treatment, subjects are asked to complete a 10 minute block of short incentivised decision tasks. While the tasks are completed individually, incentives are based on the joint performance of “social groups”, each consisting of 2 participants in the same session which are randomly matched¹, to elicit social stress. Incorrect answers and incomplete tasks are penalized. Time pressure is induced by the tight overall time limit as well as by time penalties and time limits per task. Short incentivised knowledge questions will appear on screen at seemingly random times throughout the course of the task block to induce additional stress through distractions. This stress protocol is designed to mimic stressors often experienced by low-socioeconomic mothers: making decisions with consequences for others (e.g. for the family) subject to financial and time constraints as well as distractions (e.g. by children requiring attention).

The details of the stress treatment protocol are described in the following.

i) Decision Tasks

After an initial instruction period, participants are asked to complete a block of 15 short decision tasks on the lab computers. They are given 10 minutes to complete as many tasks as they can. The expectation is that this overall time constraint is binding for a large majority of the participants and hence induces time pressure.

Participants also face individual time limits of 120 seconds for each of the 15 tasks. A countdown timer at the top of the screen indicates how much time they have left for the current task. The timer turns red after 70 seconds to indicate that time is running out and that an initial pay-off deduction (after 75 seconds – details below) is imminent. If participants have not submitted an answer after 120 seconds, their current answer is submitted automatically and the next task appears.

The decision tasks comprise budget tasks and time management tasks. For the budget tasks, participants are asked to choose the cheapest way to purchase a given basket of household expenditure items from a list of options. For example, participants might be asked to purchase 5 t-shirts choosing from a list of t-shirts which includes single items as well as value packs consisting of multiple items.

¹ Participants are made aware of the joint incentive scheme, but are unaware of their partner’s identity.

Tempo rimanente: 01:22

Ti chiediamo di trovare il modo **più economico** per acquistare **5 magliette** tra le opzioni qui sotto. Puoi scegliere sia una combinazione di diversi prodotti, sia uno o più pezzi dello stesso prodotto. Il colore delle magliette non ha importanza.

		Quantità
1 maglietta verde	7,00 €	<input type="text" value="0"/> <input type="button" value="v"/>
1 maglietta rossa	6,00 €	<input type="text" value="0"/> <input type="button" value="v"/>
1 maglietta blu	8,00 €	<input type="text" value="0"/> <input type="button" value="v"/>
Confezione risparmio: 2 magliette	10,00 €	<input type="text" value="0"/> <input type="button" value="v"/>

Figure 1: Example of a budget task

Tempo rimanente: 00:18

Ti chiediamo di trovare il modo **più economico** per acquistare **5 magliette** tra le opzioni qui sotto. Puoi scegliere sia una combinazione di diversi prodotti, sia uno o più pezzi dello stesso prodotto. Il colore delle magliette non ha importanza.

		Quantità
1 maglietta verde	7,00 €	<input type="text" value="0"/> <input type="button" value="v"/>
1 maglietta rossa	6,00 €	<input type="text" value="0"/> <input type="button" value="v"/>
1 maglietta blu	8,00 €	<input type="text" value="0"/> <input type="button" value="v"/>
Confezione risparmio: 2 magliette	10,00 €	<input type="text" value="0"/> <input type="button" value="v"/>

Figure 2: Example of a budget task after the countdown timer turns red

For the time management task, participants are given a list of diary items and are asked to schedule these in a timetable provided. The items to be scheduled are of different lengths and a variety of constraints needs to be considered when scheduling them: some items need to be scheduled at a specific time or within some given time window.

Tempo rimanente: 01:55

Attività e Vincoli	Agenda																								
<ul style="list-style-type: none"> • Aiutare con i compiti (2 ore): nel pomeriggio, dopo pranzo. • Lavoro d'ufficio (2 ore) • Portare i bambini a casa di un amico (2 ore): alle 15:00. • Lavare i piatti (1 ora) • Andare in banca (1 ora): alle 10:00. • Preparare il pranzo (1 ora): alle 11:00 	<table border="1"> <tr><td>7:00 - 8:00</td><td>Colazione</td></tr> <tr><td>8:00 - 9:00</td><td><input type="text"/></td></tr> <tr><td>9:00 - 10:00</td><td><input type="text"/></td></tr> <tr><td>10:00 - 11:00</td><td><input type="text"/></td></tr> <tr><td>11:00 - 12:00</td><td><input type="text"/></td></tr> <tr><td>12:00 - 13:00</td><td>Pranzo</td></tr> <tr><td>13:00 - 14:00</td><td><input type="text"/></td></tr> <tr><td>14:00 - 15:00</td><td><input type="text"/></td></tr> <tr><td>15:00 - 16:00</td><td><input type="text"/></td></tr> <tr><td>16:00 - 17:00</td><td><input type="text"/></td></tr> <tr><td>17:00 - 18:00</td><td><input type="text"/></td></tr> <tr><td>18:00 - 19:00</td><td>Cena</td></tr> </table>	7:00 - 8:00	Colazione	8:00 - 9:00	<input type="text"/>	9:00 - 10:00	<input type="text"/>	10:00 - 11:00	<input type="text"/>	11:00 - 12:00	<input type="text"/>	12:00 - 13:00	Pranzo	13:00 - 14:00	<input type="text"/>	14:00 - 15:00	<input type="text"/>	15:00 - 16:00	<input type="text"/>	16:00 - 17:00	<input type="text"/>	17:00 - 18:00	<input type="text"/>	18:00 - 19:00	Cena
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17:00 - 18:00	<input type="text"/>																								
18:00 - 19:00	Cena																								

Figure 3: Example of a time management task

These types of decision tasks were chosen to reflect prominent aspects of decisions faced by low-income mothers: limited financial and time resources.

ii) Distraction Pop-Ups

To induce additional stress through distractions, 10 simple knowledge questions will appear as pop-ups on screen throughout the block of tasks. The pop-ups are programmed to appear at seemingly random times within the 10 minutes, no matter what task is currently shown and how much time has elapsed on this task. When a pop-up is open, participants cannot see or continue their work on the current task until they submit an answer; however, the countdown timer for the current task is visible and continues to run down.

Tempo rimanente: 01:43

Quale dei seguenti non è un paese del Sud America?

Perù

Brasile

Argentina

Italia

Figure 4: Example of a pop-up with a knowledge question

The knowledge questions in the pop-ups are chosen such that a majority of participants will know the answer (e.g. “What is the capital of Italy?”). Stress is not induced by the difficulty of the questions, but by the interruption of the current task and the added time pressure.

iii) Incentives:

Participants in the stress treatment are randomly assigned to “social groups” of two. While participants need to complete the tasks individually, they are incentivised jointly. Each group is initially allocated 30€, this is the maximum joint incentive they can earn in the stress task block. The performance of each group member in the decision tasks and the pop-up knowledge questions determines how much of the initial 30€ the group “loses”. This joint incentive structure was chosen to induce social stress as participants feel that their choices have consequences for others. We chose to frame the incentives in terms of “losses” rather than “gains” to avoid inducing positive emotions.

Each participant can lose a maximum of 15€ to the group, 13.50€ from the decision tasks and 1.50€ from the pop-up knowledge questions.

In each of the 15 decision tasks a participant can lose up to 0.90€ to the group. There is no loss if the correct answer is submitted within 75 seconds of starting a decision task. If a correct answer is given more than 75 seconds after starting a task, 0.30€ is lost. If a wrong answer is given or a task is not attempted or completed, 0.90€ is lost.

Each of the 10 pop-up knowledge questions is worth 0.15€. If a participant gives a correct answer, there is no deduction. If a participant gives a wrong answer, 0.15€ is lost to the group.

This incentive structure ensures that participants’ performance in every single task and pop-up question will affect the group’s pay-off. This reduces the risk of participants giving up due to difficulties in solving some of the tasks.

Participants are made aware of the joint incentive structure and that they are part of a group with another participant in the same session. However, the group assignments are not announced to the participants.

e) Control Task

Instead of undergoing the stress treatment procedure, subjects in the control group are asked to complete a task which is comparable in length and of similar nature, but which is not aimed at inducing stress. Specifically, they are asked to answer 14 simple knowledge questions after reading 7 short texts about a variety of topics. The correct answers to each question can be found in the corresponding text. The questions are similar to those asked via pop-ups during the stress task.

Each text and the corresponding 2 questions are on a single page, allowing the participants to easily move back and forth between questions and texts. Subjects are given 10 minutes for this task, there are no consequences from not completing all questions. The task is not incentivised and no “social groups” are formed.

Studio dei Processi di Decisione
Istituto Universitario Europeo

Ti chiediamo di leggere questo breve testo e rispondere alle seguenti domande:

Roma, la capitale d'Italia, è una grande città multiculturale, la cui arte, architettura e cultura influenzano il mondo da quasi 3000 anni. Antiche rovine, come i Fori Imperiali e il Colosseo, evocano il potere dell'antico Impero Romano. Nella Città del Vaticano, sede della Chiesa Cattolica Romana, si possono ammirare la Basilica di San Pietro e i Musei Vaticani, che ospitano capolavori come gli affreschi della Cappella Sistina di Michelangelo.

Roma è anche il capoluogo della regione del Lazio. Roma fu costruita sopra sette colli ed è attraversata da due fiumi: il Tevere e l'Aniene. Nel 2016, Roma è stata la quattordicesima città più visitata del mondo e la più popolare attrazione turistica in Italia. Il suo centro storico, insieme alle proprietà extraterritoriali della Santa Sede e alla Basilica di San Paolo Fuori le Mura, è tra i 53 siti italiani inseriti dall'Unesco nella lista dei Patrimoni dell'Umanità.

Qual è la capitale d'Italia?

Milano	Firenze	Roma	Venezia
<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>

Qual è la sede della Chiesa Cattolica Romana?

Andorra	Città del Vaticano	Monaco	San Marino
<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>

Figure 5: Example of control task

Asking simple questions after providing the answers guarantees that participants will not experience stress due to task difficulty. Due to the lack of individual or group incentives, participants should not feel under time pressure or social stress. Furthermore, there are no interruptions creating stress.

f) Food Shopping Task

In the food shopping choice, participants are asked to use a fixed budget of 25€ to purchase grocery items in a “virtual supermarket”, a computer-based tool similar to online supermarkets. 1 out of 15 participants are randomly chosen to receive their chosen basket delivered to their home.

The details of the supermarket choice are described in the following.

i) Task Protocol & Incentives:

Participants are given 10 minutes to allocate a fixed budget of 25€ to food and drink items offered in the “virtual supermarket” tool. A variety of low-calorie and high-calorie food and drink items is available to choose from with prices matching market prices at a local supermarket. In total, approximately 150 products are on offer. Participants are encouraged to make their shopping choices as they would during a weekly shop at their local supermarket.


The supermarket choice is incentivised: 1 out of 15 participants are randomly chosen to receive their chosen basket delivered to her home approximately two weeks after the session. This incentive scheme was chosen to motivate participants to make choices representative of normal shopping behaviour.

Participants are informed that if they are selected and have not spent the entire 25€ budget, they will be paid the difference in cash up to 2€ maximum. This is to discourage non-representative shopping choices aimed at spending exactly 25€, and to ensure that participants do not feel the pressure to spend the exact amount, which could induce stress for all participants. Under this incentive scheme it is optimal for participants to aim to spend between 23€ and 25€.

ii) Choice Tool:

The “virtual supermarket” tool used to record participant’s choices has been adapted from a tool used by Spiteri et al (2018) in a previous experiment. The tool is based on a Microsoft Excel spreadsheet with VBA macros providing the interactivity necessary. When the supermarket tool is initially opened, only a “Welcome” page is visible where participants need to enter their username, password and a start code to “log in”.

Once participants have logged in, a “Supermarket” page and a “Shopping Cart” page become accessible. The “Supermarket” page contains a list of all available items. Items are grouped in 10 categories: fruit, vegetables, egg & dairy, meat & fish, bread & savoury snacks, pasta & rice, pantry, sweets, ready meals, drinks. Categories are not explicitly labelled. The order of categories and the order of items within each category is randomised.



The screenshot shows a web application titled "Supermercato Online - Excel". It displays a table of products with columns for "Prodotto", "Descrizione", "Prezzo (€)", "Quantità", and "Subtotale (€)". The table lists six items: Noverasco Pinoli 15 g, Rio Mare Tonno al Naturale 4 x 80 g, Bonduelle Mais 3 x 130 g, Carote 1 Kg, Latte UHT Scremato Microfiltrato 1 l, and Aia Durango Alette Arrosto di Pollo 0,500 kg. Each item has a small image and a price tag. The bottom of the interface shows a navigation bar with "Benvenuti", "Supermercato", and "Cestino".







Prodotto	Descrizione	Prezzo (€)	Quantità	Subtotale (€)
	Noverasco Pinoli 15 g	1.00 €	0	0.00 €
	Rio Mare Tonno al Naturale 4 x 80 g	3.89 €	0	0.00 €
	Bonduelle Mais 3 x 130 g	2.89 €	0	0.00 €
	Carote 1 Kg	1.58 €	0	0.00 €
	Latte UHT Scremato Microfiltrato 1 l	0.79 €	0	0.00 €
	Aia Durango Alette Arrosto di Pollo 0,500 kg	5.50 €	0	0.00 €

Figure 6: Virtual supermarket tool

The “Shopping Cart” page allows participants to review their choices and check how much of the budget they have spent. Once participants have completed their choice, they can submit it by clicking on a “Checkout” button. This will save a spreadsheet listing all available items and their chosen quantity for later analysis.


Cestino della Spesa					
Prodotto	Prezzo	Quantità	Totale		
Limoni 750g	1,68 €	1	1,68 €		
Mele Golden 900g	2,98 €	1	2,98 €		
Cipolle dorate 750g	0,98 €	1	0,98 €		
Latte UHT Parzialmente Scremato microfiltrato 1 L	0,95 €	1	0,95 €		
Spinacine Cotoletta con Pollo, Tacchino e Spinaci 0,220 kg	2,70 €	1	2,70 €		
Molino Bianco Pan Bauletto Integrale 400g	1,19 €	1	1,19 €		
Barilla Ditalini Rigati 500 g	0,75 €	1	0,75 €		
Heinz Tomato Ketchup 400 ml	2,29 €	1	2,29 €		
Pan di Stelle il Biscotto 350 g	1,73 €	1	1,73 €		
Molino Bianco Macine 800 g	2,79 €	1	2,79 €		
Rana Lasagne Ricotta e Spinaci 350 g	4,05 €	1	4,05 €		
Sant Anna Acqua Naturale 6 x 1,5 L	2,58 €	1	2,58 €		
TOTALE			24,67 €		
				CASSA	

Figure 7: Shopping cart of the virtual supermarket tool

g) Hypotheses

The two main hypotheses to be tested in the experiment are the following:

- 1) Acute stress leads to increased selection of foods high in calories, sugar and saturated fats, both in the context of immediate consumption (“snack choice”) and planned consumption (“food shopping choice”).
 - 1a) This dietary impact of acute stress will be stronger among mothers who cope less well with stress.
- 2) Children of mothers who experienced stress during pregnancy and who are less able to cope with stress develop less healthy food preferences.

3) Variables

a) Outcomes and Corresponding Measures:

The main outcome variables used in this study relate to the dietary choices of the mother during the experimental session and to the food preferences of her youngest child. Several measures are used to capture these rather complex concepts.

- i) Food shopping choice during the experimental session:
 - Primary outcomes: calorie, saturated fat and sugar content of the chosen basket
 - Secondary outcomes: weight of chosen fruit and vegetables
- ii) Snack intake during experimental session:
 - Primary outcomes: consumption of low-calorie snack (in grams), consumption of high-calorie snack (in grams)
 - Secondary outcomes: total calorie, saturated fat and sugar content of the consumed snacks (deterministic functions of the two outcome variables above)
- iii) Food preferences of youngest child:
 - Mediterranean Food Preference Index based on liking of food groups in *KIDMED Mediterranean Diet Quality Index for children and adolescents* (Serra-Majem et al, 2004)
 - Food groups and respective index weights: fruit (+2), vegetables (+2), legumes / pulses (+1), fish / shellfish (+1), pasta / rice (+1), cereal / grains (+1), nuts (+1), dairy products (+1), commercially baked goods / pastries (-1), sweets / candies (-1), fast food (-1), sugar-sweetened drinks (-1, additional non-*KIDMED* item)
 - Average liking of foods in each food group (based on 5-point Likert scale)
 - $MFPI = \sum_j weight_j \times AverageLiking_j$
 - BMI percentile (age & gender specific)
 - Food consumption: *KIDMED Mediterranean Diet Quality Index for children and adolescents* (Serra-Majem et al, 2004)
 - Secondary measure: Taste preferences
 - Average liking of sour, salty, umami, bitter and sweet foods
 - Sour: grapes, oranges, lemons, balsamic vinegar
 - Salty: French fries, crisps
 - Umami: mushrooms, parmesan, meat & fish foods
 - Bitter: asparagus, Brussels sprouts, black olives
 - Sweet: pastries, cake, biscuits, chocolate, gelato, cola, Fanta

b) Explanatory Variables

Explanatory variables in our study will include both the randomly assigned experimental condition and not randomly assigned factors observed or measured during the experimental session.

The randomly assigned explanatory variable is:

- i) Acute stress treatment status: stress vs control group

Explanatory variables which are not randomly assigned are the following:

ii) Chronic stress during pregnancy:

a. Depends on the mother's exposure to stressors during pregnancy and on her coping abilities.

b. Primary measures:

- Maximum and average perceived stressfulness of potentially stressful life events experienced during pregnancy
- Life events: death of close family member / friend, changes / difficulties in relationship, legal issues, change / difficulties in family life, health issues, changes / difficulties in employment of participant or spouse, financial issues, changes in habits, other potentially stressful event(s)
- Measured on 10-point Likert scale from 1="not stressful at all" to 10="extremely stressful"
- Maximum perceived stressfulness:

$$ChronicStress_{Max,i} = \begin{cases} \text{Max}(PercStress[event1]_i, PercStress[event2]_i, \dots) \\ 1 \quad \text{if no exposure to stressor} \end{cases}$$

- If experienced multiple life events: use the maximum of the perceived stressfulness scores among the events
- If no potentially stressful life events were experienced: set variable to 1 (minimum)

- Average perceived stressfulness (across all 9 events):

$$ChronicStress_{Max,i} = \frac{1}{9} \sum_{j=1}^9 PercStress[event j]_i$$

- Where $PercStress[event j]_i = 1$ if i did not experience event j

c. Validation of primary measure using variety of measures of coping abilities:

- Coping behaviour: problem-oriented, emotion-oriented, avoidance
- Perceived stressfulness of task in treatment group
- Heart rate response to stress task in treatment group:
HR response = Mean HR during stress task (10 mins) – Mean HR during baseline (5 min period at beginning of the experimental session, the period starts when the first instructions are being read out)
- Cortisol response to stress task in the treatment group:
Cortisol response = Cortisol level sample 2 – Cortisol level sample 1
- Cortisol recovery after stress task in the treatment group:
Cortisol recovery = Cortisol level sample 3 – Cortisol level sample 2

c) Control Variables

i) Hypothesis 1:

In the analysis of the dietary effects of the acute stress treatment (hypothesis 1), we will check whether the following variables are balanced across the experimental conditions and include them as control variables in the case they differ significantly between treatment and control group:

- Session time dummies
- Age of mother
- Number of children
- Marital Status
- Single Parenthood

- Education
- Household Income & Benefit Payments
- Employment status
- Dietary requirements (e.g. vegetarian / vegan, allergies, intolerances)
- Display order of snacks / supermarket products

When analysing the cortisol and heart rate responses to the stress or control task (in the context of hypothesis 1), we control for the following factors which are known to have a considerable effect on the levels and reactivity of cortisol and heart rate:

- Time since waking up
- Time since last menstrual period (dummies for different stages of menstrual cycle)

We furthermore check whether the following factors are balanced across groups and control for them if they differ significantly:

- Time since last food intake, big meal, consumption of cocoa product, liquid intake, alcohol consumption, caffeine intake, medication intake, physical exercise
- Dummy indicating whether participant is a smoker / number of cigarettes smoked per day
- Temperature in laboratory
- Allergies
- Regular medical treatment / medication
- Use of oral contraceptives
- Onset of menopause
- Endocrine disorder

ii) Hypothesis 2:

In the analysis of the effects of stress during pregnancy on children's food preferences (hypothesis 2), we control for the following variables:

- Age of youngest child
- Gender of youngest child
- Mother's Education
- Household Income & Benefit Payments
- Current diet of mother: *Index of Mediterranean Diet Adherence* based on *Validated 14-item Questionnaire* by Martínez-González et al (2012)
 - Scoring of the index and the thresholds are the same as in Martínez-González et al (2012)
 - Questions were adapted to clarify serving sizes and to avoid "leading" questions (Yes/No questions). In some cases the questions were converted from daily to weekly consumption frequencies to better incorporate thresholds of "< 1 serving per day".
- Diet of mother during pregnancy: Adapted *Index of Mediterranean Diet Adherence* based on *Validated 14-item Questionnaire* by Martínez-González et al (2012)
 - Adaptation to pregnancy setting: Positive index item for wine consumption is removed.
- Chronic stress of mother during past 3 months
 - Perceived stressfulness of potentially stressful life events experienced in past 3 months (computed as outline above for the pregnancy period)

- Perceived stress during past 3 months: average score of 2 questions from the Perceived Stress Scale (PSS) questionnaire (Cohen et al, 1983):
 - felt nervous and stressed
 - could not cope with things

We will furthermore check whether the following variables vary significantly with our explanatory variable and control for them if this is the case:

- Age of mother
- Number of children
- Marital Status
- Single Parenthood
- Employment status
- Dietary requirements (e.g. vegetarian / vegan, allergies, intolerances)

d) Variables Relating to Potential Mechanisms:

The following variables will be used to explore potential mechanisms in the effects of acute stress on dietary choices:

- Time preferences (generally and in health dimension)
- Risk attitudes (generally and in health dimension)
- Dutch Eating Behaviour Questionnaire (DEBQ) – Dimension for Emotional eating

4) Empirical Strategy

To test the hypotheses outlined above, we estimate linear models using the outcomes and explanatory variables described in the previous section. Initially, we will estimate models of the following form:

$$Y_i = \sum_{k=1}^K \beta_k T_{ki} + \varepsilon_i$$

where Y_i denotes the outcome for participant i and T_{ki} are explanatory variables, either based on the experimental treatment or based on not randomly assigned factors measured during the experiment. β_k are the coefficients of interest and ε_i is an idiosyncratic error term.

More augmented specifications will include a vector of control variables X_i :

$$Y_i = \sum_{k=1}^K \beta_k T_{ki} + X_i' \gamma + \varepsilon_i$$

These augmented specifications are particularly relevant in the case of not randomly assigned explanatory variables.

To account for potential error correlation among individuals in the same experimental session, we will estimate standard errors robust to clustering at the session level. Due to the relatively small number of clusters, the wild cluster bootstrap approach proposed by Cameron et al (2008) will be used to estimate the clustered standard errors.

a) Impact of mothers' acute stress on immediate food consumption ("snack choice") and planned food consumption ("food shopping choice"):

- i) Dependent variables (primary outcomes):
 - a. Snack intake during experiment
 - b. Average calorie, sugar and saturated fat content of shopping basket
- ii) Explanatory variables:
 - a. Acute stress treatment status: stress vs control group
- iii) Basic specifications:
 - a. Separate bivariate linear model for each dependent variable
- iv) Specifications with interaction of stress treatment status with:
 - a. Coping abilities of mothers:
 - Coping behaviour: problem-oriented, emotion-oriented, avoidance
 - Perceived stressfulness of task²
 - Heart rate response to task²
 - Cortisol response to task²
 - Cortisol recovery after task²
 - b. Emotional eating score based on the DEBQ

² These measures only capture coping abilities among the stress treatment group.

- v) Further specifications:
 - a. Include pre-treatment control variables that differ significantly across groups
 - b. Explore potential mechanisms by including measures of time preferences and risk attitudes
- vi) Comparison between the effects on immediate and planned consumption can provide further insights into the mechanisms linking acute stress to food choices, i.e. the role of food cravings and impaired decision making respectively.
 - a. If the effect of acute stress on immediate consumption is larger than that on planned consumption, this would point to a stronger relevance of temporary food cravings as a mechanism.
 - b. If on the other hand the effect of acute stress on planned consumption is larger, this would point to impaired decision making due to cognitive overload as a relevant mechanism.
- vii) Check the effectiveness of the stress protocol:
 - a. Primary measure: Perceived stressfulness of stress task (compared to control group)
 - b. Secondary measures:
 - 1) Heart rate response to stress task (compared to control group)
 - 2) Cortisol response to stress task (compared to control group)

Both heart rate and cortisol are subject to influences other than stress. Effects on heart rate and cortisol are furthermore difficult to observe as the timing of the measurements is of crucial importance. Perceived stressfulness is hence the primary measure used to check the effectiveness of our stress protocol.

b) Impact of mothers' chronic stress during pregnancy and of their coping abilities on children's food preferences:

- i) Dependent variable: food preferences of youngest child
 - a. Primary measures:
 - Mediterranean Food Preference Index
 - BMI percentile
 - Food consumption: *KIDMED* index
 - b. Secondary measures:
 - Taste Preferences
- ii) Explanatory variable: Chronic stress during pregnancy
 - a. Primary Measures:
 - Maximum and average perceived stressfulness of potentially stressful life events experienced during pregnancy
 - b. Validation of primary measure using variety of measures of coping abilities:
 - Coping behaviour: problem-oriented, emotion-oriented, avoidance
 - In stress treatment group only:
 - Perceived stressfulness of task
 - Heart rate response to stress task
 - Cortisol response to stress task
 - Cortisol recovery after stress task

iii) Basic specifications:

- a. Separate bivariate linear model for each measure of food preferences and the primary measure of chronic stress during pregnancy

iv) Further specifications:

- a. Include the control variables outlines above. Controlling for potentially confounding factors is important here since the explanatory variable is not randomly assigned.
- b. Validation of primary measures of chronic stress during pregnancy using measures of coping abilities: examine correlations between primary measures and measures of coping abilities.

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