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Science & Technology in childhood Obesity Policy

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D8.2: The More and Less Europe study, a randomized controlled trial (RCT) for overweight and obesity in pre-schoolers: Report on design of the RCT, recruitment, measurements, staff training and intervention delivery

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Dissemination Level		
PU	Public	x
PP	Restricted to other programme participants (including the Commission Services)	
RE	Restricted to a group specified by the consortium (including the Commission Services)	
CO	Confidential, only for members of the consortium (including the Commission Services)	

Abbreviation	Definition
App	Application
BMI	Body mass index
CEBQ	Child Eating Behaviour Questionnaire
CFPQ	Comprehensive feeding practices questionnaire
DNA	Deoxyribonucleic acid
GIT	Gastrointestinal tract
GLP	Glucagon-like peptide
HIF3A	Hypoxia-inducible transcription factor 3A
1H-NMR	Proton nuclear magnetic resonance
KEEP	Keeping foster and kin parents supported and trained
mHealth	Mobile health
ncRNA	Non-coding Ribonucleic acid
miR	Micro Ribonucleic acid
MINISTOP	Mobile-based intervention intended to stop obesity in pre-schoolers
ML	More and Less
MCCV-PLS-DA	Monte Carlo cross-validated partial least square-discriminant analysis
PCA	PCA, principal component analysis
PCR	Polymerase chain reaction
PLS-DA	Partial least square-discriminant analysis
PYY	Peptide YY
RCT	Randomized controlled trial
RIA	Radioimmunoassay
ROC curves	Receiver operating characteristic curves

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1 Summary

1.1 Background

Childhood overweight and obesity is a serious public health issue with an increase being observed in preschool-aged children. Treating childhood obesity is difficult and few countries use standardized treatments. Therefore, there is a need to find effective approaches that are feasible for both health care providers and families. Thus, the overall aim of this study is to assess the acceptance and effectiveness of a parent support programme (the More and Less, ML) for the management of overweight and obesity followed by a mobile health (mHealth) programme (the MINISTOP application) in a socially diverse population of families in three European sites.

1.2 Methods/design

A two-arm, parallel design randomized controlled trial in 300 2-to 6-year-old children with overweight and obesity from Romania, Spain and Sweden (n=100 from each). Following baseline assessments children are randomized into the intervention or control group in a 1:1 ratio. The intervention, the ML program, consists of 10-weekly group sessions which focus on evidence-based parenting practices, followed by the previously validated MINISTOP application for 6-months to support healthy eating and physical activity behaviors. The primary outcome is change in body mass index (BMI) z-score after 9-months and secondary outcomes include: waist circumference, eating behavior (Child Eating Behavior Questionnaire), parenting behavior (Comprehensive Feeding Practices Questionnaire), physical activity (ActiGraph wGT3x-BT), dietary patterns (based metabolic markers from urine and 24hr dietary recalls), epigenetic and gut hormones (fasting blood samples), and the overall acceptance of the overweight and obesity management in young children (semi-structured interviews). Outcomes are measured at baseline and after: 10-weeks (only BMI z-score, waist circumference), 9-months (all outcomes), 15- and 21-months (all outcomes except physical activity, dietary patterns, epigenetics and gut hormones) post-baseline.

1.3 Discussion

This study will evaluate a parent support programme for weight management in young children in three European countries. To boost the effect of the ML programme the families will be supported by an app for 6-months. If the programme is found to be effective, it has the potential to be implemented into routine care across Europe to reduce overweight and obesity in young children and the app could prove to be a viable option for sustained effects of the care provided.

2 Introduction

According to the World Health Organization childhood obesity is one of the gravest public health challenges of today's society (1), with approximately 108 million 2- to 19-year-old children being classified as having obesity (2). More specifically, in children less than five years, there has been a swift increase in childhood overweight and obesity and if these trends continue it is predicted that 70 million children will be overweight or obese by 2025 (3). These statistics are concerning as Geserick et al. (4) found that 90% of 3-year-olds with obesity still had overweight or obesity in adolescence. Furthermore, for those adolescents with overweight or obesity, the majority of weight gain happened between two and six years of age (4). Thus, this demonstrates the need for evidence-based treatment programs in the pre-school years in order to attempt to rectify the increased prevalence of childhood overweight and obesity.

According to Colquitt et al. (5) for children under six years of age multicomponent interventions (i.e., diet, physical activity, and behavioral interventions) seem to be effective at treating overweight and obesity. However, the authors did state that evidence is limited (5). To date, the majority of the treatment interventions for overweight and obesity use face-to-face delivery methods (6). A recent meta-analysis by Ling et al. (6) found small effect sizes on treatment interventions for preschool-aged children for body mass index (BMI) (-0.28 kg/m^2, $p< 0.001$) using various in person delivery methods. However, the More and Less (ML) study found that at the 12-month follow-up, a 10-week group treatment programme focusing on parenting practices had a greater reduction in BMI z-scores than standard treatment in health care (-0.30 vs. -0.07, $p<0.05$) (7, 8). An even greater reduction was observed in the intervention group who received booster sessions (a 30-minute phone call every 4 to 6 weeks over a 9-month period) (7, 8). These results are promising; however, sustained contacts with families after treatment programs are burdensome on both health care providers and participants, which makes it difficult to scale-up. Therefore, different types of boosters need to be used in order to reduce the burden on both health care and participants.

The universal use of smartphones makes the use of mobile health (mHealth) an option for boosting the effects of treatment programs. mHealth is increasingly being used for promoting healthy habits and as treatment of many types of health conditions and diseases. In adults, two meta-analyses have found that mHealth interventions focusing on weight loss significantly decreased participants' weight in the intervention groups compared to the control groups (9, 10). In children and adolescents few studies have utilized mHealth in the prevention or treatment of obesity (11-15) and hardly any have been conducted in the preschool-age group (16, 17). The Mobile-based Intervention Intended to Stop Obesity in Preschoolers (MINISTOP) trial was a mHealth obesity prevention intervention that was developed and led by Marie Löf and her team to improve 4-year-old children's body composition, dietary, physical activity, and sedentary behaviors (18, 19). The MINISTOP intervention had a significant effect on a composite score composed of body composition, diet, and physical activity variables, with this effect being more evident among children with a higher fat mass index (19). There are numerous advantages of mHealth over conventional intervention approaches such as: the programs can be delivered any time and place; are interactive; can be tailored to different groups (e.g., translated into multiple languages); and reduces burden on health care professionals and participants. These advantages further motivate the use of mHealth in families with young children with overweight and obesity.

The mechanisms that drive weight gain such as epigenetics and gut hormones are still unclear (20, 21). Epigenetics has received attention during the recent years as it may be implicated in the transmission of obesity risk between parents and offspring and in the heritable regulation of gene expression, without altering their coding sequence (22). The most relevant epigenetic mechanisms

involved in gene activity control are histone modifications, non-coding RNAs (ncRNA) and DNA methylation (21). Further, obesity has been associated with the epigenetic modulation of several genes. For example, a relationship has been reported between increased BMI and adiposity as well as higher DNA methylation levels at the hypoxia-inducible transcription factor 3A (HIF3A) gene (23). Moreover, an increased methylation in the gene RXRA measured at birth has been associated with greater adiposity in later childhood (24). Two other investigations identified a strong correlation between obesity and serum levels of micro RNA (miR)-122 and miR-519d (25) and found DNA methylation to be related to insulin resistance (26). However, these findings need to be confirmed and further explored in young children.

Another field of interest for obesity is the gastrointestinal tract (GIT) (27). The GIT plays an important role in acute appetite regulation through a number of mechanisms: (1) the release of hormones that play a role in appetite regulation such as anorectic hormones (Peptide YY, PYY, and glucagon-like peptide, GLP-1) and orexogenic gut hormones (e.g., ghrelin), (2) the enteric nervous system and signals through the vagus to the brain to influence appetite and (3) secondary to stimulating signals from other organs such as liver adipose. Previous research in adults has demonstrated that the infusion of the GIT anorectic hormones PYY and GLP-1 at physiological doses has profound effects on the suppression of appetite (27). Also, weight loss appears to lead to a suppression of PYY and GLP-1 suggesting a role in feelings of hunger during weight reduction. However, evidence of the role of GIT hormones in overweight and obesity among young children is sparse.

A major challenge in the management of obesity in both adults and children is understanding what people eat. Most dietary assessment methodologies use methods of self-reported food intake which are subject to large misreporting error (28, 29) leading to bias in the measurement of child diet. Garcia et al developed a new metabolomic methodology for dietary assessment using urine, which is not subject to the same misreporting errors (30). This method has been validated in adults. Our aim is to apply the same method to the dietary assessment of children.

To the best of our knowledge there is no study to date that has attempted to assess a broad array of key biological and social determinants of obesity in young children. Here we report the outlines of the design of a multi-country study that incorporates both a parent group support programme and mHealth in an overweight and obesity intervention in 2- to 6-year-old children with overweight and obesity. Parts of this protocol have already been published (31)

2.1 Aim

The overall aim of this study is to assess the feasibility, acceptance and effectiveness of an overweight and obesity intervention in a socially diverse population of families. The specific aims are:

1. To determine its effectiveness on child weight status (BMI z-score) via of a 10-week parent support programme delivered in groups focusing on evidence-based parenting practices (the ML program) followed by a mHealth component for 6-months (the MINISTOP application, app) for overweight and obesity in preschool-aged children.
2. To assess change in secondary outcomes, which are: waist circumference, child eating behavior, parental feeding practices and physical activity.
3. To assess epigenetic mechanisms and physio-pathological processes underlying childhood obesity, including the role of gut hormones.
4. To assess and validate child food intake with metabolic markers in urine metabolomics.
5. To evaluate the feasibility of recruitment (facilitators and barriers), attrition and acceptability of the ML program, the standard treatment and the overall acceptance of overweight and obesity management according to patients and care providers.

2.2 Hypotheses

Our central hypothesis is that the intervention (the ML programme followed by the MINISTOP app for boosting) will be more effective in decreasing children's BMI z-score (primary outcome), food habits and behaviors, and physical activity (secondary outcomes) compared to standard care. Another study hypothesis is that the intervention will produce changes in urinary metabolites, which will serve as biomarkers of the nutritional outcomes or as targets for application. We also hypothesize that the parent programme and the mHealth intervention will be well accepted by families and caregivers.

3 Methods

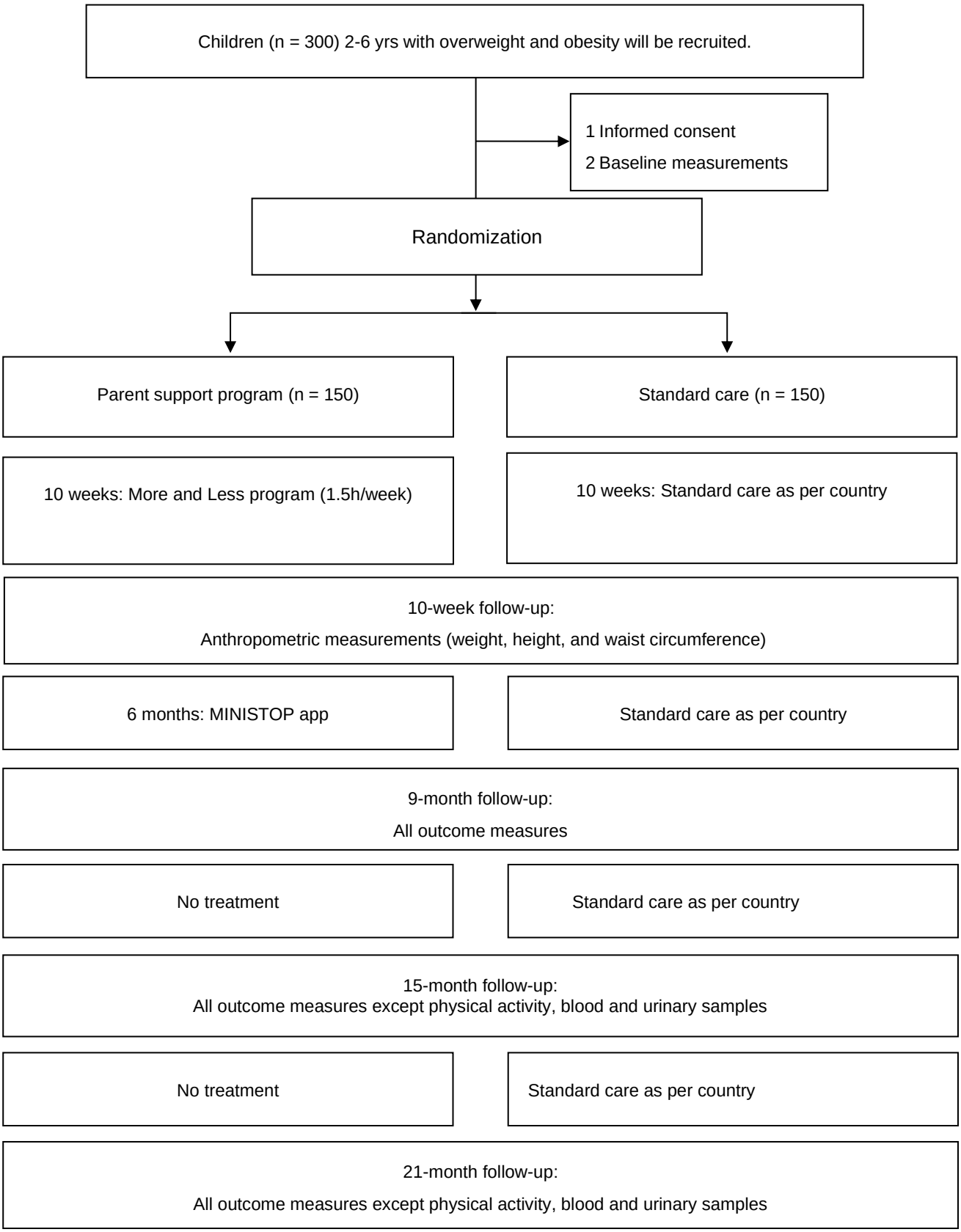
3.1 Study design

ML Europe is a two-arm parallel design randomized controlled trial (RCT) comparing overweight and obesity treatments in 2-6-year-old children in three countries (Romania, Spain, and Sweden). Following baseline assessments, participants will be randomized into the intervention and control group in a 1:1 ratio. The intervention group receives a 10-week parent group treatment programme (the ML program) which focuses on evidence-based parenting practices (7, 8) followed by a previously validated 6-month mHealth app (the MINISTOP app, PI: M Löf) to support healthy lifestyle changes (18, 19). The control group receives standard treatment as offered in the country of participation. It should be noted that the standard care varies in each of the three countries. The different interventions are described in greater detail below. Assessments will be conducted at 10 weeks, 9 months, 15 months, and 21 months post-baseline (see **Figure 1** for study outline).

3.2 Sample size and power calculation

Based on power calculations, 75 children are needed in each group (adjusted for drop-out) per site to detect a difference of 0.3 BMI z-score with 85% power at the 9-month follow-up between the intervention and control group. These calculations are based on a previous study in this age group (32). Thus, each site aims to recruit 100 participants to ensure adequate power.

Figure 1. Flow-chart of the More and Less Europe trial design.



3.3 Participants, eligibility, and recruitment

In total, we aim to include 300 families (n = 100 in Romania, Spain, and Sweden, respectively). See an update on the recruitment process under Trial status. To be included in this study: children must be between 2-6 years old and have overweight or obesity as classified by international cut-offs (33); have no other underlying medical condition(s); the child has not started any treatment for overweight or obesity; and at least one parent has to have the ability to communicate in Romanian, Spanish, or Swedish depending on the country of participation. Parents who do not own a smartphone compatible with the MINISTOP app will be excluded from this study (i.e., version 10.0 or higher for iOS or version 5.0 or higher for Android). Families that are asked about participation but never referred to us will not be included in the analyses of non-participation; however, we will be able to compare participating families to those who are referred to the study but reject participation before randomization.

Recruitment will follow a standardized protocol for all countries. See additional efforts made to speed up recruitment under Trial Status. In Romania, family physicians and pediatricians hand out information regarding the study to families with 2- to 6-year-olds with overweight or obesity. Parents who want to learn more about the study are provided with a phone number, email address, web page and Facebook page with information of how to contact the research group. Participants can also be recruited, as self-referrals, using an official page for the study on Facebook to be shared with specialized groups.

In Spain, families with children who attend weight and height assessments at their pediatricians at primary care health centers and hospitals are asked to participate in the study. If the parents are interested in participating, the pediatrician will schedule a visit within a maximum of seven days to provide them with more detailed information regarding the study and for them to sign the informed consent.

Finally, in Sweden, the recruitment methods have been previously described in detail (7, 8). Briefly, recruitment is done primarily at primary child health care centers, where all parents of children from birth to 5 years of age are offered free, yearly check-ups. If overweight or obesity is detected the nurse provides a verbal and a short one-page explanation of the study. If the parent(s) are interested in participating the nurse sends a referral to the research group that will send out more detailed information regarding the study together with a consent letter. After one week, a member from the research team will contact the families to answer any questions that they have. Recruitment is also conducted at secondary health care (i.e., out-patient pediatric clinics). Additionally, self-recruitment is being done through newspaper ads as well as by placing posters on primary health care bulletin boards.

For all countries, after fully informing the families, if they still want to participate, they send back the signed consent letter, which is subsequently signed by a member of the research team and a copy is sent back to the family. A time for baseline assessments is then scheduled with the research group.

3.4 Randomization and blinding

After the consent form has been signed, the participants are randomly allocated to either the intervention group (parent support programme and mHealth booster) or the control group (standard care as per country) at a 1:1 ratio via a random allocation sequence list (in blocks of three). The sequence list was generated using free software environment for statistical computing and graphics R (version 3.5.1) (34). The random allocation sequence is managed by a person who has no relationship with recruitment or treatment and opaque envelopes are used to ensure concealment.

Those assessing the outcomes are blinded to the treatment allocation; however, owing to the nature of the intervention participants are not blind to their allocation.

3.5 Intervention

The More and Less Program

The ML programme is based on the Keeping Foster and Kin Parents Supported and Trained (KEEP) parenting program, which has been tested in multiple settings (35-38). KEEP is based on Bandura's Social Learning Theory (39) and Patterson's Social Interaction Learning Theory (40, 41). The key concept of the programs is to support parents in evidence-based parenting practices, especially regarding positive reinforcement and limit setting, in order to improve parent and child communication. In ML, the improved communication lays the foundation for parents to support a healthy lifestyle for the child.

The ML programme is composed of 10 weekly sessions (1.5 hours/week) and access to the Swedish manual that was adapted from the US context during the original ML study (7, 8, 42). The manual has now been culturally adapted for Romanian and Spanish families with preschool aged children with overweight or obesity. Adaptations were specifically made to text referring to national guidelines and recommendations regarding healthy lifestyles (food, diet and physical activity), and to add examples of traditional and local recipes. Adaptations were also made to material handed out to parents to follow information for local healthcare and educational settings.

Table 1 displays the content of the ML programme (7). Beyond the evidence-based parenting practices, the programme includes content regarding healthy food habits, physical activity habits, as well as techniques to help parents regulate emotional control. Each session begins with a theoretical introduction to parenting skills, the focus of the session is then discussed and practice is done through role play and homework assignments. To facilitate the implementation of the ML programme it follows a manual where the sessions are described with precise instructions to the group leaders (2 per group). The parents receive a manual which summarizes what has been discussed during each session. For parents who are unable to attend sessions, the parental manual is sent home to the family and the family is contacted by phone for a brief review of the session.

Staff training and supervision

The ML group leaders received an initial four-day training in child overweight and obesity management and in the ML programme content. The training was held in Romania, 1-4 December 2018 (see **Supplement 1** for the content) and provided by the ML programme developers PN and AE. The teams from Romania and Spain attended. During the training, the participants received lectures on basic elements of overweight and obesity management and the theoretic background of the ML program. The sessions of the programme were then thoroughly discussed and practiced. The group leaders were trained in how to deliver the programme by acting as group leaders while the other participants acted as parents according to the role scripts that the participants received before the meeting. Throughout the training, cultural differences and adaptations were brought up and discussed such as food culture, time for scheduled group sessions (in Spain it is commonly accepted that people arrive late for scheduled meetings) and differences in limit setting strategies in the three countries. After the training, the manuals were further revised in collaboration with all partners. The training of group leaders was continued by external supervision after each weekly session for the first group in all countries during 2019. See the Trial Status below. The group leaders were asked to watch the filmed sessions and reflect on how they delivered the programme. In Sweden and in Spain, groups have been held in health care facilities and in Romania in university facilities.

Table 1. Session content of the More and Less programme.

More and Less Parenting Program	
Session	Content
1	Welcome and overview
2	Food and play: When more? When less?
3	Parents as teachers: cooperation and energy balance
4	Parents as teachers: to teach children new behaviors
5	Rewards and incentives
6	Pre-teaching
7	Parents as teachers: limit setting strategies
8	Power struggles: to avoid and to handle them
9	More support – Less stress
10	Summary: parenting, food and play – to prepare for the future

The MINISTOP app

The MINISTOP app was developed and evaluated in a population-based study with preschool aged children (PI: Marie Löf) and has been previously described in detail (18, 19). Briefly, MINISTOP is composed of an extensive programme of information and push notifications built using current guidelines for a healthy diet and physical activity in pre-school aged children (43). Over the 6-month period 12 themes will be covered (**Table 2**). A new theme is introduced bi-weekly, with parents being alerted by a push notification when this happens. Every theme is split into three parts (general information; advice; and strategies to change unwanted behavior). Through the app, parents have the ability to register their child's consumption of sugar sweetened beverages, candy, fruits and vegetables, and physical activity and sedentary behavior. Parents then receive feedback on the registered parameters at the end of every week. Reminder messages are sent out to parents if they have not opened the app after a couple of days (18).

In ML Europe, the app was first translated to English and then to Romanian and Spanish. Similar cultural adaptations were then made to the app as those described for the ML program, such as national guidelines for food and diet and examples of recipes to match the foods most commonly eaten in each country. Two days before the tenth and final session of the ML program, parents receive an email with a username and password for the MINISTOP app as well as a text message with a link to download the app. At the final session, the ML programme leaders will ensure that all parents were able to download the app and sign in. Thereafter, they will explain how the app works to the parents and answer any questions that they may have.

Table 2. Themes included in the MINISTOP app.

MINISTOP app	
Theme	Content
1	Healthy foods in general
2	Breakfast
3	Healthy small meals
4	Physical activity and sedentary behavior
5	Candy and sweets
6	Fruits and vegetables
7	Drinks
8	Eating between meals
9	Fast food
10	Sleep
11	Foods outside the home
12	Foods at special occasions

3.6 Control

The weight management offered to the control group follows the standard care procedure for each country of participation. In Romania and Spain, the control group receives an evaluation of a one-day food frequency questionnaire as well as a 30-minute consultation with a doctor that is a specialist in childhood nutrition, where healthy lifestyle recommendations are made. The parents also receive a hand-out which provides general recommendations for healthy food and physical activity in 2- to 6-year-olds. Furthermore, in Romania the children are re-evaluated after 3 months during a 15-minute consultation. In Sweden, the control group receives standard care according to the Action plan for overweight and obesity for Stockholm County (44). Children with overweight and children with obesity younger than 4 years receive support from their child health care nurse. Children older than 4 years with obesity are followed in an outpatient pediatric clinic with yearly visits to a pediatrician and follow-up visits to a pediatric nurse, approximately 5 visits (30 minutes in duration) per year (8). The treatment centers around supporting the family in creating healthy diet and physical activity habits for the child. Children may also be referred to dieticians, psychologists or physiotherapists.

3.7 Measures

Outcome measures are collected at baseline, 10 weeks, 9, 15 months, and 21 months post baseline. Table 3 presents when outcome measures are assessed and the instruments used to assess child and parental behaviors are displayed in Table 4.

3.7.1 Primary outcome

BMI z-score is the primary outcome measure which is the most commonly used indicator of weight change in pediatric obesity studies (45). The children's weight and height will be measured to the nearest 0.1 kg and 0.1 cm, respectively. A fixed stadiometer is used to assess height and weight will be measured with the children wearing only underwear. BMI is derived as weight (kg) divided

Table 3. Socio-demographic characteristics and outcome measures collected at different time points.

Outcomes	Measure	Baseline	10 weeks	9 months	15 months	21 months
Child						
Weight and height (BMI z-score)	Measured by health care professionals	x	x	x	x	x
Waist circumference		x	x	x	x	x
Date of birth	Child background questionnaire	x				
Country of birth		x				
Gender		x				
Health status		x		x	x	x
Family structure		x		x	x	x
Daycare		x		x	x	x
Visits to health care regarding weight		x		x	x	x
Screen time		x		x	x	x
Breakfast consumption		x		x	x	x
Sugar sweetened drinks consumption		x		x	x	x
Eating behavior	Child Eating Behavior Questionnaire	x		x	x	x
Physical activity / sedentary behavior	ActiGraph wGT3x-BT accelerometer	x		x		
Food intake	Urine samples, 24hr dietary recall	x		x		
Gut hormones	Fasting blood samples	x		x		
Epigenetic markers	Fasting blood samples	x		x		
Parent						
Weight and height (BMI)	Parent background questionnaire	x		x	x	x
Date of birth		x				

Country of birth		x			
Gender		x			
Education level		x			
Health status		x	x	x	x
Occupation status		x	x	x	x
Income		x	x	x	x
Social and economic support from network		x	x	x	x
Perceived level of comfortable life		x	x	x	x
Parenting behavior	Comprehensive Feeding Practices Questionnaire	x	x	x	x

Abbreviations: BMI, body mass index

by height (m) squared. BMI z-scores are then calculated using age and gender specific reference values (33).

Table 4. Measures used in the study

Instrument, reference	Domains	No. items	Description
Child eating behavior questionnaire (CEBQ),		35	
Wardle et al. 2001	*Food approach*		
	Food responsiveness	5	The child's general appetite
	Enjoyment of food	4	The child's interest in food
	Emotional overeating	4	If the child eats as a response to emotions
	Desire to drink	3	The child's desire to drink
	Food avoidance		
	Satiety responsiveness	5	If the child gets full easily or not
	Slowness in eating	4	The child's speed of eating
	Emotional undereating	4	If the child eats less in response to emotions
	Fussiness	6	The child eats a limited variety of food
Comprehensive feeding practices questionnaire (CFPQ),		49	
Musher-Eizenman & Holub 2007	Monitoring	4	Parents keep track of child's intake of less healthy foods
	Emotional regulation	3	Parents use food to regulate the child's emotional stress
	Food as a reward	3	Parents use food as a reward for child behaviour
	Child control	5	Parents allow the child control of his/her eating behaviors and parent-child feeding interactions
	Modelling	4	Parents actively demonstrate healthy eating for the child
	Restriction for weight	8	Parents control the child's food intake with the purpose of decreasing or

		maintaining the child's weight
Restriction for health	4	Parents control the child's food intake with the purpose of limiting less healthy foods and sweets
Teaching nutrition	3	Parents use explicit didactic techniques to encourage the consumption of healthy foods
Encourage balance and variety	4	Parents promote well-balanced food intake, including the consumption of varied foods and healthy food choices
Pressure to eat	4	Parents pressure the child to consume more food at meals
Healthy environment	4	Parents make healthy foods available in the home
Involvement	3	Parent's encourage child's involvement in meal planning and preparation

3.7.2 Secondary outcomes

Waist circumference

Waist circumference is measured at the mid-point between the lower rib and iliac crest to the nearest 0.1 cm using a non-elastic tape measurer.

Eating behavior

The children's eating behavior is assessed using the Child Eating Behavior Questionnaire (CEBQ) (46). It includes 35 items on eating styles comprising eight factors related to the risk of obesity. Parents rate each behavior on a five-point Likert scale (`never´, `rarely´, `sometimes´, `mostly´, and `always´ for items 1 to 13 and `disagree´, `slightly disagree´, `neutral´, `slightly agree´, and `agree´ for items 14 to 49). Mean scores for each sub-scale are calculated. This questionnaire has been found to have high internal reliability and good validity (46-52).

Parenting behavior

The Comprehensive Feeding Practices Questionnaire (CFPQ) is used to measure parenting behavior (53). The CFPQ is a parent-report instrument, designed to measure feeding practices of parents of children aged 2-8 years. It contains 49 items comprising 12 factors, where parents rate each behavior on a five-point Likert scale (`never´, `rarely´, `sometimes´, `mostly´, and `always´). The CFPQ has previously been validated in Brazilian preschoolers (54).

Physical activity and sedentary behavior

The ActiGraph wGT3x-BT accelerometer (ActiGraph Corp, Pensacola, USA, www.actiGraphcorp.com) is used to assess physical activity and sedentary behavior over seven consecutive 24 hour periods. The ActiGraph will be attached the child's non-dominant wrist and be worn at all times, except for water-based activities (e.g., showering/bathing or swimming). The recorded movements will be used to estimate time in various activity levels based on appropriate cut-points.

Metabolites of food intake

First void urinary samples will be collected from the children and will be used to assess metabolites of food intake. Two urine samples from the child will be collected by the parents at home six and three days before the visit to the research group. The third urine sample is collected on the morning of the visit to the research group. The urine metabolite analysis will be carried out as previously described (30). In brief, urine samples will be measured by proton nuclear magnetic resonance (^{1}H-NMR) spectroscopy. Global urinary ^{1}H-NMR profiles will be used to predict the quality of the diet using the World Health Organization guidelines as a reference. Individual urinary metabolites associated with the intake of foods will be used to assess the dietary profile of the child. The Dietary Metabotype Score that embodies concentrations of urinary metabolites related to food components and adherence to diet will be developed and validated against one 24-hour dietary recall with a parent. The 24-hour recall will cover the day before the visit to the research group. For children attending preschool a food diary for teachers to fill out will be collected to cover the food intake not provided by the parent.

Epigenetic markers and gut hormones

Fasting blood samples are collected to assess reversibility of metabolic markers through epigenetic markers and the role of gut hormones.

Epigenetic markers

The epigenetic analysis is carried out in white blood cells, which require DNA extraction, bisulphite transformation and analysis with Polymerase chain reactions (PCRs) or other technologies involving hypothesis driven methylation (CpGs). The unit of measurement/criteria is the change in percentage CpGs. The methodology has been explained in detail elsewhere (55, 56). Methylation levels will be analyzed following standardized epigenetic methods after bisulphite conversion as described previously (55, 56) in hypothesis- driven specific CpGs.

Gut hormones

PYY concentrations will be measured using an in-house radioimmunoassay (RIA). The assays are highly sensitive and do not cross-react with other gut hormones. Separation of the antibody-antigen complexes from the free antigen is achieved by secondary antibody. The reported intra and inter-assay variation is 5.8% and 9.8% respectively.

GLP-1 concentrations will be measured using an in-house RIA. This assay is highly specific and sensitive with the antibody cross reacting with 100% of all amidated forms of GLP-1. The assay does not cross react with glycine extended forms (GLP1-37 and GLP9-37) or any other gut hormones. The lowest level of GLP-1 that can be detected by this assay is 7.5pmol/l. Separation of the antibody-antigen complexes from the free antigen is achieved by charcoal adsorption. The reported in-house intra- and inter-assay variation is 5.4% and 11.5% respectively.

Feasibility, attrition, and acceptability of the ML intervention

Using semi-structured interviews, the facilitators and barriers of recruitment as well as attrition to the intervention (first 10 weeks, i.e., the ML parent programme) and feasibility and acceptability of the standard care offered are assessed. Both parents and healthcare professionals are interviewed by trained research staff. During the interviews a set of questions are asked to all participants follow-up questions are however based on individual responses. The questions have been tested in pilot interviews with both parents and health care professionals. The interviews are recorded and fully transcribed. Interviews will be conducted before and after the intervention. See Trial status and The impact of Covid-19 for a description of the qualitative studies that have been conducted.

Sociodemographic data

At baseline parents are asked to fill out a background questionnaire for the child and themselves. Questions for the parent include: health status, sociodemographic factors and social support. For the child, questions include: country of birth, health status, family structure and lifestyle related questions such as food and screen time behaviors.

3.8 Adverse events

Adverse events will be monitored, reported and handled appropriately. The risks imposed by this research project are deemed to be low, i.e., the burden of the experiments for the research subjects is limited. It is important to note that blood samples collected in the study are optional and not a criteria for participation. However, blood samples are taken by experienced nurses and a pain reducing cream is used to reduce any discomfort. Urinary samples are none invasive and thus cause no risk to the participants. In addition, the investigators have extensive experience conducting behavioral weight control studies, and active efforts will be taken by the research staff to ensure the participating families' safety. Other adverse events may include psychosocial burden that parents may experience when made aware about their child's weight status and the sense of guilt that may arise. To mitigate this, the causes and consequences of overweight and obesity are reviewed in a non-judgmental way in the first session of the ML programme. Also, to reduce the potential impact on the child's self-esteem, preferred ways to talk about body weight and obesity with children, if necessary, are addressed.

3.9 Statistical analysis

Intention-to-treat analysis using generalized linear mixed models with repeated measures will be used to examine the effects of the intervention on primary (aim 1) and secondary outcomes (aim 2) for the total study population (i.e., all three sites). The link function for the primary outcome (BMI z-score) is identity and the distribution of the dependent variable assumed to be Gaussian (equivalent to a linear regression). In secondary outcomes we will use a Gaussian identity and family link function for waist circumference, physical activity and sedentary behavior, and a logarithmic link and Poisson family function (equivalent to a Poisson regression) for child eating behavior and parental feeding practices. A random effect for country will be used to account for the clustered study design. In the models, we will control for relevant covariates such as gender, age, parental weight status, education level, income and foreign background. A random intercept and random slope for time will be included in the model to control those non-observed confounders specific to each child that could be constant or vary in time, respectively. Furthermore, interactions between variables will be estimated. If missing values in the outcomes (primary and secondary) are more than 10%, these will be imputed through a two-part model (also known as a model for semi continuous data). In this model, we would simultaneously estimate the probability of not being missing (first part) and the outcome (second part), using a mixed generalized linear model, in which we would include, as explanatory variables:

age, gender, parental weight status, foreign background, educational level, and the random effects which are aforementioned.

Statistical tests and analyses of the interaction of phenotypical outcomes with epigenetics will include Manhattan plots, volcano plots, principal component analysis (PCA)/cluster, heatmaps, partial least square-discriminant analysis (PLS-DA), correlations and association studies, linear regression models, receiver operating characteristic (ROC) curves and these will be implemented as appropriate.

The means and medians for the gut hormone values before and after the intervention will be compared, using Student's t test and Mann-Whitney U test, respectively. The differences will be adjusted in a generalized linear mixed model, with an identity link and Gaussian family, including the confounders, both observed and unobserved, indicated above.

For the validation of child food intake with metabolic markers in urine, the urinary dietary model will be derived using previously described methodology (30). Comparison between the study groups will be carried out using PCA and Monte Carlo cross-validated partial least square-discriminant analysis (MCCV-PLS-DA) methodology. The relationship between dietary biomarkers and the dietary metabolite profile will be carried out using a generalized linear mixed model, with an identity link and Gaussian family, including, again, the confounders.

The semi-structured interviews with parents and health care professional will be fully transcribed verbatim and analyzed using thematic analysis (57).

For our analyses we will use R (34), STATA version 12.1 (StataCorp 2011, College Station, TX, USA) and SPSS Statistics (IBM, Armonk, NY, USA).

3.10 Ethics approval

This trial was approved by: the Ethics Committee of Scientific Research in University of Medicine and Pharmacy “Victor Babes” Timisoara (25/31.10.2018), the Balearic Islands Ethics Committee (IB 3814/18 PI), and the Research Ethics Committee, Stockholm, Sweden (2018/2082-31/1). Written informed consent is obtained from all parents/caregivers.

3.11 Trial status

During the autumn in 2018 we applied for ethical permission for the RCT to be conducted in all three study sites. While waiting for ethical permission we translated the Swedish group leader and parental manuals of the ML parent programme to English and then to Spanish and Romanian. When the translations and cultural adaptations (described above) were finished, we scheduled a four-day group leader training, this was conducted in December 2018 in Timisoara, Romania (See supplement 1 for training content). Twelve potential group leaders from both Spain and Romania attended the training. In Sweden, the group leaders attended their training in September 2018.

Six group leaders, 2 from each country, have now been certified in the ML parent programme after attending weekly supervision during their first parent group. The Romanian group received supervision during the spring/summer 2019 and the teams in Sweden and Spain during the autumn in 2019. The group leaders recorded the group sessions and were then asked to watch the recordings after each session and reflect on how they delivered the programme. What went well? What was challenging? and What would they like feedback on during supervision? The group leaders wrote down their reflections that were then used as a guide to supervise the teams.

3.11.1 Recruitment

In Sweden and Romania recruitment began in January 2019 and Spain began to recruit in February 2019. Recruitment has been challenging from the start. First, the start of the STOP project was delayed to June instead of February as originally planned in the grant proposal. This had major implications for recruitment as the ethics applications could not be submitted until the autumn due to holiday breaks that started in Sweden in late June. Consequently, the recruitment could not start until December 2018 - February 2019 (site dependent) as in some countries, the ethics approval process took longer than in others due to differences in ethics processes. A further challenge in Stockholm were structural changes in the health care section. Many large outpatient pediatric clinics closed down, which affected the number of children that were referred to us from these clinics. The recruitment is now further delayed due to the Covid-19 pandemic. The recruitment was originally expected to end 2020 but will now be extended to end 2021. The following efforts have been made in each study site to speed up recruitment. In Romania, family physicians and pediatricians hand out information regarding the study to families with 2- to 6-year-old children with overweight or obesity. All (n=246) family physicians from Timisoara were contacted via email and informed about the programme. Approximatively 20 pediatricians were also informed about the programme. Parents who want to learn more about the study are provided with a phone number, email address, web page and Facebook page with information on how to contact the research group. Participants can also be recruited, as self-referrals, using an official page for the study on Facebook to be shared with specialized groups. In addition, to speed up recruitment we have extended information to another city (Resita) where city hall and kindergartens have provided support for dissemination. 114 children were suitable to be included in the programme, however only 7 families responded and were enrolled. We had 4 onsite visits (in Resita) for recruitment. So far, one parent group has been conducted and one more was planned for the spring 2020.

In Spain, families with children who attend weight and height assessments at their pediatrician in primary care health centers and hospitals are asked to participate in the study. If the parents are interested in participating, the pediatrician will schedule a visit within a maximum of seven days and provide them with more detailed information regarding the study and an informed consent form. If word of mouth provided new participants, the same protocol was also applied to these participants. To increase the flow of participants to the study the research group has conducted a questionnaire study to be able to understand the barriers and facilitators for health care professionals to recruit families to the study. The study was inspired by the interview study conducted in Sweden (58). As one of the reasons for families not to sign up to the study was that they had to travel between cities

within the island to be able to participate in parent support group sessions. Thus, the groups are now offered in three different sites (one in the capital town, Palma, in the south/west of the island; a second one in the west/middle, Marratxí; and the third in the east, Manacor). At present, 29 pediatricians and 16 pediatric nurses are involved in the recruitment. One parent group was conducted during the autumn, 2019, and two had to be suspended this spring.

In Sweden, all primary child health care centers in Stockholm County (approx. 120) are informed about the study and the approx. 500 child health care nurses working there are reminded on a regular basis to refer children to the study. The research group then send out more detailed information regarding the study together with a consent letter. After one week, a member from the research team contacted the families to answer any questions that they had. Recruitment is also conducted within secondary health care (i.e., out-patient pediatric clinics). Additionally, as advertisement of the study has been done through newspaper ads as well as by placing posters on primary health care bulletin boards, self-referrals are also possible. In addition, the research team at KI has been interviewed in a podcast organized by the Child Health Care Services in Stockholm and widely visited by Swedish health care professionals. WP leader and team members have also been interviewed in both national television, radio and newspaper about childhood obesity and here acknowledged the recruitment of families to the study. KI had organized a conference in March to support health care professionals in how to talk to families with a young child with overweight or obesity to help speed up recruitment. However, this conference had to be suspended and is now planned for September.

To facilitate participation in the study, the measurements and the intervention is carried out in clinical settings close to where the families live in two different sites in Stockholm. One parent group has been conducted and two had to be suspended during the spring. To speed up the delivery of the intervention after the summer we plan two parallel groups during autumn 2020.

As part of the qualitative assessment of the project, to understand facilitators and barriers for families to participate in the study, the research group has interviewed both health care professionals (58) and parents (Master thesis: by C. Neovius. Parents experiences of conversations concerning children´s weight in the child health care setting. KI. 2020). The findings reveal that overweight and obesity in preschoolers is a sensitive matter for both parent and child health care nurses to talk about. Still, the parents reported how they wanted information about their child's weight to be able to help their child. The nurses requested better training in how to raise the weight issue to parents in a better way as well as a better organization for what the health care sector could offer these families. The findings have been communicated to the units that refer children to the study to encourage them to recruit children to the study. We are now writing a manuscript summarizing the parents' experiences of conversations regarding their child´s weight in the child health care setting that we aim to submit before the summer.

3.11.2 The impact of Covid-19

The Covid-19 virus situation across Europe has led to a suspension of the randomized controlled trial within in the STOP-project. The suspension will delay the recruitment, data collection and delivery of the intervention. If we are able to continue with the study as planned and start the intervention again by September/October 2020, the data collection will be delayed by an additional six to 12 months. We will be able to report on primary outcomes after 9 months for most of the families; however, the long-term follow-up at 15 and 21 months will be extended beyond the final date of the STOP project. In **Figure 2,** please find the current recruitment and data collection situation for the trial in Romania, Spain and Sweden. In the figure legends we describe how the data collection

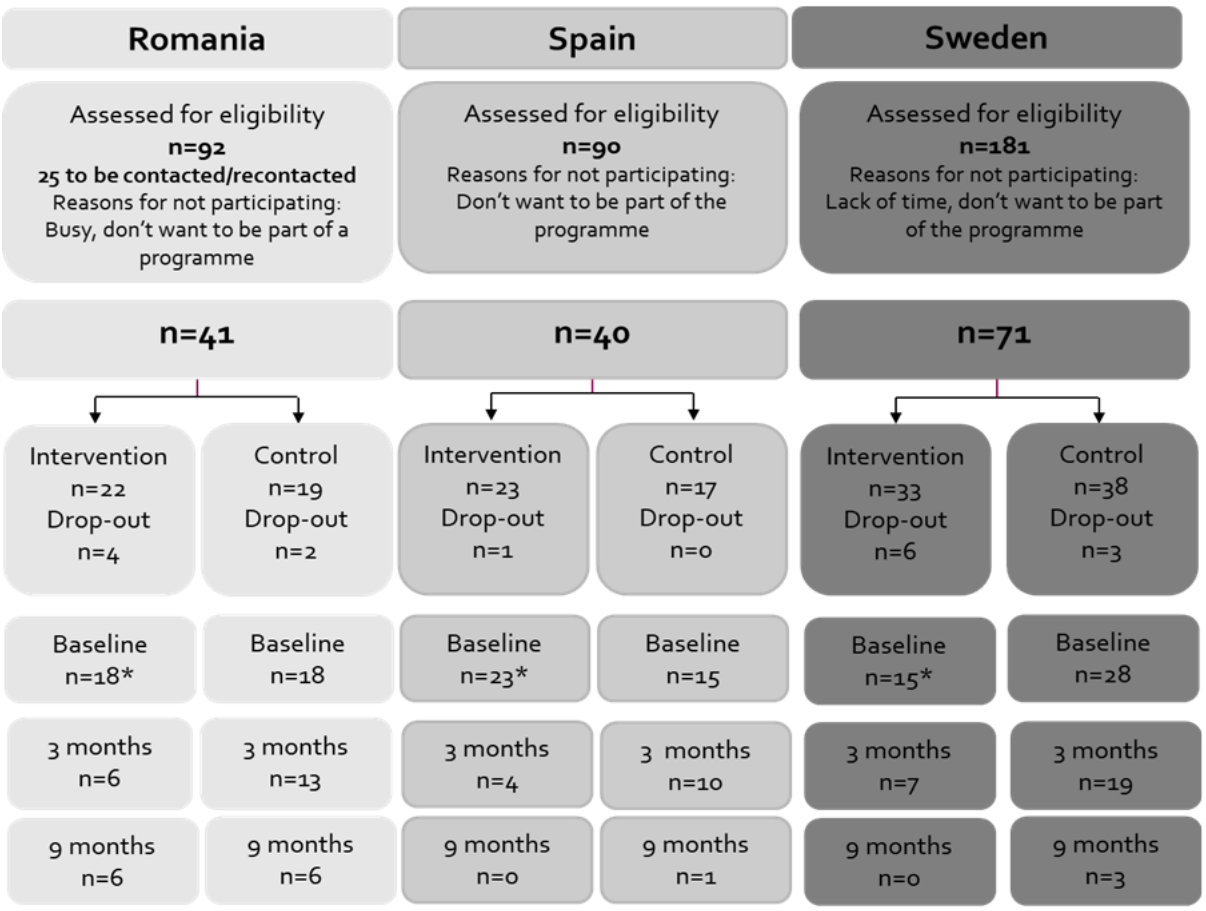
is affected. During the current situation when families are unable to participate in treatment and measurements the following actions are being made in each study site:

In Sweden, we have conducted interviews with parents that are participating in the trial. We are now writing a manuscript summarizing the reports from the first interviews, as described above. Additionally, we are now conducting further telephone-based interviews with parents in the study. The aim of these interviews is to examine how the Covid-19 situation has affected their family's life and habits regarding food and physical activity/inactivity as well as contact with the healthcare providers regarding their child weight development. The questions were piloted with three parents before the final draft of the interview guide. This guide has been translated to English so that the Spanish and Romanian teams can conduct the same interviews in their populations. Together, these qualitative efforts will provide a broader picture of how the pandemic has affected the everyday life of families with children with overweight and obesity in Europe. The interviews will also provide data on how the different actions taken to handle the Covid-19 pandemic in the three countries, will have affected the physical and psychosocial health in these families.

In Spain, inspired by the qualitative study performed by the team at KI (58), the research team has conducted a similar study using questionnaires, where the asked pediatricians of their perceptions and experiences of addressing young children weight to parents and what they perceived as barriers for families to participate in child weight management. The Spanish research team are now conducting interviews regarding how the Covid-19 pandemic has affected the Spanish families participating in the study.

In Romania, interviews with pediatricians have been conducted to get their perceptions and experiences of addressing young children's weight status with parents and what they perceive to be the barriers for families to participation in child weight management programmes. These interviews are now being analyzed. The results will be summarized in a manuscript before submission. Like the Swedish and Spanish teams, the Romanian team are also conducting interviews with study participants regarding the Covid-19 pandemic situation.

Figure 2. Recruitment status in May 2020



Notes: Drop-out=those who have been randomized but then decided not to participate.
*Includes children that have been measured for baseline but the group was cancelled and thus may have to be repeated. See below.

Measures that are affected by coronavirus - will have to be repeated (baseline) or delayed (3 and 9 months)

Romania
Baseline: n=8
3 months: n=3 (June)
9 months: n=6 (August, September)

Spain
Baseline: n=10
3 months: n=12 (March n=3, April n=1, May n=8)
9 months: n=10 (May n=1, June n=9)

Sweden
Baseline: n= 8
3 months: n=7 (April n=2, May n=2 and June n=3)
9 months: n=17 (April n=3), May n=7 and June/July n=7)

4 Discussion

The ML Europe trial will assess the impact of parent group sessions (the ML program) followed by a mHealth application (the MINISTOP app) to treat overweight and obesity in 2- to 6-year-old children from three European countries. Globally, there have been very few overweight and obesity treatment interventions targeted at pre-school aged children (6) and to date, no intervention has coupled face-to-face delivery with mHealth to boost the effect of the intervention.

4.1 Strengths and limitations

Study population and recruitment

In this trial we aim to recruit a representative sample of the study population in each participating country. In Sweden this will be done by inviting all primary and secondary health care centers in Stockholm County to participate in recruitment, with a similar process being done in Spain (all primary health care centers and hospitals in Mallorca were invited to participate). However, the ability to get a representative sample of the study population in Romania might be more difficult as recruitment relies on families contacting the research team themselves through contacts with physicians and pediatricians and Facebook announcements. Therefore, certain parts of the population may be missed, e.g., those not likely to contact the research team and those who do not use Facebook.

Additionally, there are a few other factors that should be considered with regards to recruitment, which have the possibility to influence the representativeness of the overall sample. Firstly, the participating families need to be able to understand, speak, and read Romanian, Spanish, or Swedish sufficiently well (depending on the country of participation) in order to participate. Secondly, families with low socioeconomic status and parents with a lower educational background have been found to be less likely to participate in research (59, 60). The inability to speak the language the intervention is being conducted in coupled with the possibility of low participation rates in families with low socioeconomic status is of concern. This is due to the fact that children of migrant parents and those of low socioeconomic status are more likely to have overweight or obesity (61, 62). Furthermore, families will only be included if they own a smartphone compatible with the MINISTOP app, which could affect recruitment of low socioeconomic families; however, we believe this risk to be quite small as smartphones are so commonly used in most populations. Finally, we did foresee that recruitment for this study would be a challenge as was found in the ML trial (8). In the ML trial parents decided not to participate for various reasons, with the most common being parents' work schedules or family situation (8). When recruiting for ML Europe we use our experience from previous clinical RCTs to ensure that recruitment and patient participation are organized in the most feasible way, e.g., time, date and place for the parent groups will be adjusted to suit as many families as possible. We anticipate the recruitment to be influenced by target population size which varies between the countries (330 000 in Timisoara, 860 000 in Mallorca and 2.3 million in Stockholm County). Also, we are aware that the prevalence of overweight and obesity among children differs in each site. While recent national data are yet to be published, in Romania, a study including 6-year-old children found the prevalence for overweight and obesity to be 19% (63). In Spain, the prevalence of overweight and obesity was 21% in 3- to 5-year-old children (64). In the Stockholm County, the prevalence of overweight and obesity among 4-year-old children is on average 11% ranging from 4% in the more affluent areas to over 15% in less affluent areas (65). Thus, although the prevalence of obesity seems to be lowest in the Swedish site the larger population may compensate this

challenge. It remains to be elucidated what the largest barrier in the recruitment process will be: not sufficiently large targeted population or low prevalence of overweight and obesity.

When comparing the effects of the intervention between countries, it should be borne in mind that the standard care in the control group varies between countries. This variation may alter the average treatment effect and it is likely, for example, that the treatment effect will be lower in Sweden

Study design, intervention, and control

The randomized controlled design and multi-site recruitment (i.e., Timisoara, Romania; Mallorca, Spain; and Stockholm, Sweden) are strengths of this study. Furthermore, the fairly large sample size (n = 300) will allow us to assess the intervention's effectiveness in samples within and across three very different European countries. With regards to the intervention, both components are based on behavior change theories (i.e., Bandura's Social Learning Theory (39) and Patterson's Social Interaction Learning Theory (40, 41) for the ML programme and Social Cognitive Theory (66) for the MINISTOP program). Furthermore, the combination of group sessions followed by a previously evaluated mHealth app is a further strength, as it will allow for the reiteration of the material taught during the group sessions to be explained in different ways with different examples. This is important as the booster group in the ML study had a mean change in BMI z-score from baseline which was significantly larger in comparison to standard treatment and the group without boosters (-0.54, $p<$ 0.001; -0.11, p =0.551; and -0.04 for the booster, without boosters, and standard treatment groups, respectively) (8). In today's society, telephone-based booster sessions after an intervention such as ML are difficult to sustain due to parents' busy schedules. Therefore, an mHealth solution such as MINISTOP to booster the effect of treatment may be a more feasible approach as it allows parents to work through the material at their own pace, when they have time. Finally, this study is limited by the fact that there is no standard overweight and obesity treatment across Europe. Therefore, the control group will receive different treatment depending on the country of participation, which could influence the results. However, standard treatment as per country is the best possible control as it would be considered unethical to withhold treatment for a condition if a treatment exists (67).

While obesity is more prevalent among girls at age 4 years (68), by age 10, obesity rates are similar across genders (69). This shows that early obesity treatment is equally important for boys and girls. However, regardless of age, overweight and obesity disproportionately affect socially disadvantaged children (68-70). We have addressed this disparity in the ML Europe study, in which we encourage the participation of families from lower SES and migrant backgrounds. We focus our recruitment in areas with high obesity prevalence and offer childcare to participants during the parent group. In the original ML trial (8), our recruitment efforts were successful: among the parents, 60% were of migrant background and without higher education (8). Moreover, the ML programme is designed to address the additional challenges and treatment barriers lower SES families face. Previous group participants have highlighted the program's discussions about parental stress and practical tips about affordable healthy foods and family activities as particularly helpful (42).

4.2 Outcome measures

The use of objective assessments for anthropometrics and body composition, physical activity and sedentary behavior, food intake, as well as epigenetic and metabolic markers is a further strength of this study. Additionally, the use of qualitative methods, i.e., semi-structured interviews with health care professionals and parents from all sites will allow us to assess the feasibility of this new overweight and obesity management intervention in three European countries.

4.3 Conclusion

In the majority of European countries, there is no standard management of overweight and obesity in the pre-school years. As overweight and obesity in this age group may track into adolescence and adulthood, causing psychological and physical consequences, families should receive support as early as possible. Feasible and effective approaches for families with pre-school aged children are yet to be developed. If the ML Europe intervention is found to be effective, it has the potential to be implemented into routine care for overweight and obesity across Europe.

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Author contributions

All authors were involved in the study design for More and Less Europe. AE is the project coordinator for the three sites and drafted the manuscript together with CDN who also aided in the development of the MINISTOP app's content. ACE is the primary investigator for the Romanian site and JAT is the primary investigator for the Spanish site. Regarding recruitment and data collection KN is the coordinator in Sweden, CP in Romania and EA and CB in Spain. JAM is responsible for analyses of epigenetic and metabolic markers and their interpretations. GF and IGP are responsible for the analyses of gut hormones and validation of food intake through urine. MS is responsible for statistical analysis. ML created the original MINISTOP programme and led the work when it was modified for ML Europe. All authors read and approved the final manuscript. PN is responsible for the Swedish site and the primary investigator of the ML Europe.

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Overweight and obesity in young children
Programme Sunday 2 December 2018, kl. 08:30-17:00

Room: Piata Eftimie Murgu no 2 floor 2, code 300041, Timisoara

Lecturers: Paulina Nowicka, Prof., Adela Chirita, Assistant Prof. and Anna Ek, postdoctoral fellow

- 08:30-09:00 Introduction to the day and presentation of the group
- 09:00-10:00 Update on prevalence, causes and consequences
- 10:00-10:15 Break
- 10:15-11:15 Prevention and treatment – evidence and gaps of knowledge
- 11:45-12:30 Lunch
- 12:30-13:00 Physical activity
- 13:00-13:45 Parenting & social relations
- 13:45-14:00 Coffee break
- 14:00-14:30 Example of a conversation: Lisa 6 years
- 14:30-15:15 More and Less study and More and Less Study Europe – design, results and experiences
- 15:15-15:25 Short break
- 15:25-16:30 Experiences and advice regarding successful recruitment
- 16:30-17:00 Summary and questions

Training workshop in the More and Less Method

Monday 3 Dec 201	Tuesday 4 Dec 2018	Wednesday 5 Dec 2018
08:30-08:40 Introduction to the day and the workshop	08:30-09:00 Introduction to the day with time for questions	08:30-10:00 Session 8 incl coffee break *Theme: Power struggels*
08:40-10:30 Session 1 incl coffee break *Theme: Welcome och overview Parents' 4 Key Roles*	09:00-11:00 Session 5 incl coffee break *Theme: Charts and incentives*	10:00-10:20 Break
10:30-10:40 Break	11:00-11:15 Break	10:20-12:00 Session 9 *Theme: More support – less stress*
10:40-11:30 Session 2 (Part 1) *Thene: Food and Play: When more? When less?*	11:15-12:15 Session 6 (Part 1) *Theme: Parents as Teachers: Pre-teaching and planning*	12:00-12:30 Lunch
11:30-12:15 Lunch	12:15-13:00 Lunch	12:30-14:00 Session 10 incl coffee break *Summary and preparations for the future*
12:15-13:00 Session 2 Cont.	13:00-14:00 Session 6 Cont.	Time for site-specific preparations. Paulina and Anna leave for the airport
13:00-13:10 Break	14:00-14:10 Break	
13:10-15:10 Session 3 incl coffee break *Theme: Parents as Teachers. About collaboration*	14:10-16:10 Session 7 incl coffee break *Theme: Limit Setting Strategies*	
15:10-15:20 Break	16:10-16:20 Break	