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D3.5: Report on causal models, including the identification of their actionable segments, into policy work packages

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Science and Technology in childhood Obesity Policy

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Dissemination Level

PU	Public	☒
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
STOP	Science & Technology in childhood Obesity Policy
NINFEA	Nascita ed INfanzia: gli Effetti dell'Ambiente
IOTF	International Obesity Task Force
WHO	World Health Organization
RRRs	relative risk ratios
mtDNA	mitochondrial DNA
EWAS	epigenome- wide association study
MWAS	Metabolome-wide association scans
FDR	False discovery rate
BCAA	branched chain amino acid
DAGs	Direct acyclical graphs
TE	Total effects
NDE	natural direct effects
NIE	natural indirect effects
NIEM1	natural indirect effect via the first mediator
NIEM2	natural indirect effect via the second mediator
DMRs	differentially methylated regions
HELIX	Human Early-Life Exposome
ARIES	Accessible Resource for Integrated Epigenomics Studies
UPF	ultra-processed food
VLDL	very large density lipoproteins
IDL	intermediate density lipoproteins
HDL	high density lipoproteins
LDL	low-density lipoproteins
PCA	principal component analysis
DSA	Deletion/substitution/addition
NDVI	Normalized Difference Vegetation Index
zBMI	Z score BMI (according to the WHO Child Growth Standards)
zHeight	Z score height (according to the WHO Child Growth Standards)
zWeight	Z score weight (according to the WHO Child Growth Standards)
ACDSi	Analysis of Children's Development in Slovenia
CRO-PALS	Croatian Physical Activity in Adolescence Longitudinal Study
MVPA	moderate and vigorous physical activity per week

WP3 D3.5 Update December 2021

The present update complements the original deliverable 3.5 released in 2020 and includes a response to the comments raised by the EC assessors concerning translation into policy and comments from members of the STOP ISAB.

Exposome research on childhood obesity: suggestions for policy

Introduction

Childhood overweight and obesity are increasing in most of the world, and this trend hampers the health of future generations, since obesity in childhood leads to poor ageing and an increased risk of chronic diseases in adulthood. Programmes to prevent childhood obesity have been so far mainly school-based, and effects have been limited, with best results obtained in younger children. Such programs have almost entirely focused on behavior-oriented prevention. Non-behavioural recommendations include physical activity at school, taxation of unhealthy foods, and standards for meals at kindergartens and schools. Also, breastfeeding is recommended (WHO, 2017).

The aim of this deliverable is to identify causal pathways that are supported by reasonably sound evidence, are biologically plausible and are actionable, to be able to guide prevention of obesity in children. The update is based on systematic reviews of the literature, and on new evidence at multiple levels, from geographic investigation of distal determinants of obesity (spatial analysis) to proximal individual behaviours (including physical activity and dietary habits) down to intermediate molecular changes. This approach corresponds to the principles of “exposome”, i.e. considering a multiplicity of relevant exposures, encompassing characteristics of the environment, individual behaviours and intermediate biological mechanisms such as metabolic and epigenetic changes.

We consider consistency across the different layers of evidence, and potentially actionable endpoints to formulate some policy proposals that also take social disparities into account.

1 Systematic reviews

1.1 Risk factors

We focus on risk factors for childhood obesity at two general time-periods: early life, with a focus on prenatal exposures, and those risk factors during childhood and adolescence that constitute the “obesogenic environment”.

For early-life risk factors, we build upon background knowledge coming from previous epidemiological studies on the main risk factors for children obesity in the first 1,000 days of life. A systematic review conducted by others (Baidal et al., 2016) examined 282 studies that met the inclusion criteria. Several risk factors during the first 1,000 days were consistently associated with later childhood obesity. These included *higher maternal pre-pregnancy BMI, prenatal tobacco exposure, maternal excess gestational weight gain, high infant birth weight,*

and accelerated infant weight gain. Fewer studies also supported gestational diabetes, child care attendance, low strength of maternal–infant relationship, low socioeconomic status, curtailed infant sleep, inappropriate bottle use, introduction of solid food intake before age 4 months, and infant antibiotic exposure as risk factors for childhood obesity. In the following analyses we have considered the most relevant of these risk factors.

1.2 External exposome: built environment and childhood obesity

We systematically reviewed the available evidence on characteristics of the built environment and their link to childhood obesity (Obesity Reviews, in press 2021). The focus was on environmental factors such as traffic noise and air pollution, as well as physical factors potentially driving obesity-related behaviours, such as neighbourhood walkability and availability and accessibility of parks and playgrounds. We identified epidemiological evidence via the online databases Ovid MEDLINE, Embase, and Web of Science, using site-specific combination of MeSH terms and keywords with no time restrictions. Included studies were i) conducted in children below the age of 18 years, ii) focused on body size measurements in childhood, iii) examined at least one built environment characteristic, iv) reported effect sizes and associated confidence intervals, and v) were published in English language. The articles screening, data extraction and quality assessment were conducted independently by two reviewers. To assess the quality of the studies we used a modified version of the Newcastle-Ottawa scale tailored to cohort studies, including cross-sectional studies. A *z*-Test, as alternative to the meta-analysis, was used to quantify associations due to heterogeneity in exposure and outcome definition. Our search initially identified 1192 studies with some studies included in more than one built environment domain. Following a rigorous screening based on our eligibility criteria, we included 4 studies on traffic noise, 14 studies on air pollution, 19 studies on neighbourhood walkability and 28 studies on availability and accessibility of parks and playgrounds in our review. Studies were generally of high quality with 42% longitudinal studies. Strong evidence was found for an association of NO_2/NO_x exposure (p<0.001), street intersection density (p<0.01), and access to parks (p<0.001) with childhood obesity. Our systematic review indicates an effect of some characteristics of the built environment on childhood obesity, mainly related to traffic-related air pollution and characteristics supporting walking. We identified a lack of studies which account for interactions between different built environment exposures or verify the role and mechanisms of important effect modifiers such as age.

An overview of the evidence on the external exposome is reported in the following table, where the evidence has been rated according to strength. None of the risk factors associated with the built environment have a level of evidence greater than limited.

Table 1 Relative strength of the association between urban environmental exposures and health associations in children(Gascon, Vrijheid, & Nieuwenhuijsen, 2016)

	Fetal growth restriction	Obesity and related outcomes	Neuropsychological development impairment (cognitive function and behavior)	Respiratory/immune effects
Street connectivity, house density, and walkability	No studies	Inadequate	No studies	No studies
Food environment	No studies	Inadequate	No studies	No studies
Green spaces	Limited	Limited	Inadequate	Inadequate
Outdoor air pollution	Sufficient	Inadequate	Limited	Sufficient
Noise	Inadequate	Inadequate	Inadequate	Inadequate
Extreme temperature	Inadequate	No studies	No studies	Inadequate

Sufficient, if most of the studies, including good quality studies, report an association, but evidence is not yet conclusive enough to conclude that there is a causal relationship; Limited, several good quality, independent, studies report an association, but evidence is not yet conclusive enough; Inadequate, emerging evidence for an association based on some studies; Evidence for lack of association, several good quality studies are consistent in showing no causal relationship; No studies, no studies have been conducted

[a]Results are mainly based on sleep disturbance related to ambient noise

In addition to this published review, within WP3 we also evaluated the epidemiological evidence on the built environment and its link to childhood obesity, focusing on environmental factors such as traffic noise and air pollution, as well as physical factors potentially driving obesity-related behaviours, such as neighborhood walkability and availability and accessibility of parks and play grounds. We found strong evidence for an association of traffic-related air pollution (nitrogen dioxide and nitrogen oxides exposure, $p < 0.001$) and built environment characteristics supportive of walking (street intersection density, $p < 0.01$ and access to parks, $p < 0.001$) with childhood obesity (Malacarne et al, 2021).

1.3 Internal exposome: molecular pathways

We considered also underlying molecular and metabolic pathways, and to this end we conducted two systematic reviews. The first is a systematic review of metabolomic studies of childhood obesity, following the PRISMA guidelines (Handakas et al, Obesity Reviews, in press 2021). We searched across Scopus, Ovid, Web of Science and PubMed databases for articles published from Jan 1 2005 to Jul 8th 2020. We retrieved 1,271 records, and finally retained 41 articles for qualitative synthesis. Study quality was assessed using a modified Newcastle–Ottawa Scale. 33 studies were conducted on blood, six were conducted on urine, three were conducted on umbilical cord blood, and one was conducted on saliva. 30 studies were primarily cross-sectional, five studies were primarily longitudinal, and seven studies examined effects of weight-loss following a life-style intervention. A consistent metabolic profile of childhood obesity was observed including *amino acids (particularly branched chain and aromatic amino acids), carnitines, lipids and steroids.* These signatures appear largely concordant with those in adult studies (Rangel-Huerta, Pastor-Villaescusa, & Gil, 2019).

Although the use of metabolomics in childhood obesity research is still developing, the identified metabolites can provide additional insight into the pathogenesis of many obesity-related diseases, hough longitudinal studies are lacking (Handakas et al, 2021).

A second STOP review was conducted on epigenetic markers (Alfano et al, Obesity Reviews, in press 2021). The literature search was performed via Pubmed and Scopus engines using a combination of terms related to epigenetics and pediatric obesity. All articles studying the association between epigenetic mechanisms (including DNA methylation and hydroxymethylation, non-coding RNAs and histone modification) and obesity and/or overweight (or any related anthropometric parameters) in children (0-18 years) were included. 121 studies were selected. DNA methylation was the most widely investigated mechanism (N=101 studies), followed by non-coding RNAs (N=19 studies) with evidence suggestive of an association with childhood obesity for DNA methylation of specific genes and specific miRNAs. One study was identified for histone modifications. However, high heterogeneity of the findings was noted and no strong causal inferences can be drawn. The temporal sequence between epigenetic changes and onset of childhood obesity is uncertain because epigenetics may be altered by a wide range of stimuli, including metabolic changes associated with obesity itself. Available evidence is stronger for DNA methylation and supports adiposity in childhood leading to changes in methylation, rather than the other way around, consistently with findings in adults (Sun et al., 2019; Wahl et al., 2017). If obesity causes epigenetic changes, then epigenetics may fall on the causal pathway between obesity and obesity related outcomes, as already suggested in children (Reed, Suderman, Relton, Davis, and Hemani (2020) and in adult studies (Campanella et al., 2018). Interaction between the different epigenetic layers is still based on assumptions rather than solid scientific evidence.

Overall, a conclusion of the systematic reviews is that molecular or metabolic research currently does not make consistent contribution to policy in terms of interventions to prevent obesity.

New findings from the STOP consortium

2. External exposome: built environment, physical activity and anthropometric measurements

2.1. Built environment and obesity risk factors in the Helix consortium

The Helix consortium is based on six existing European birth cohorts (BIB, EDEN, KANC, MOBA, Rhea and INMA). The present analysis is based on 1,581 children (unpublished). We hypothesised that different aspects of the urban environment influence child physical and sedentary activity levels and performed an “exposome-wide association study” of various built-environment indicators to highlight the most influential.

We have investigated:

- Population density (number of inhabitants/km^2),
- Building density (m^2 built/km^2) within a buffer of 300m,
- Connectivity density (number of intersections / km^2) within a buffer of 300m;

- Access to public transport in terms of lines (meters of bus public transport mode lines inside each 300m buffer),
- Access to public transport in terms of stops (number of bus public transport mode stops inside each 300m buffer);
- Facility density (number of facilities present divided by the area of the 300m buffer);
- Facility richness (number of different facility types present divided by the maximum potential number of facility types (at a 300m buffer),
- Mixed land use (Land use Shannon's Evenness Index)
- Walkability index (as mean of deciles of facility richness index, land use Shannon's Evenness Index, population density, connectivity density)
- Total traffic load of major roads in 100m buffer;
- Total traffic load of all roads in 100m buffer;
- Traffic density on nearest road;
- Inverse distance to nearest road
- Average of Normalized Difference Vegetation Index (NDVI) values within a buffer of 100m;
- Green and blues space within 300m
- Green distance: Straight line distance to nearest green space > 5,000m^2;

Outcomes were based on questionnaires and included: moderate to vigorous physical activity (validated with accelerometer data in a subset), extracurricular physical activity, sedentary behaviours, TV viewing, computer / video games, other sedentary behaviours, sleep, and daily time of active transport (walking and biking). To study the lifestyles in combination, we performed a principal component analysis (PCA) of the lifestyle outcomes. We first examined each exposure independently by linear regression models, and models were adjusted for cohort, maternal education, family affluence score, area level SES, child sex and age (with correction for multiple comparisons). We then performed a Deletion/substitution/addition (DSA) method to build multiple exposure models. Indicators of exposure to green spaces near home (i.e. Normalized Difference Vegetation Index, NDVI, a measure of vegetation (i.e. plant cover) and presence of green spaces) were inversely associated with sedentary behaviours (overall and specific activities), showing more green space to be associated with less sedentary behaviour ($p < 0.05$). On the other hand, population density was associated with sedentary behaviours overall and specifically with higher television viewing ($p < 0.05$). Higher traffic load of major roads at home area was associated with less sleeping time in childhood. Similar estimates were obtained in the multiple exposure models, except for building density that was associated with a decrease in sedentary time.

Higher vegetation both in home and school areas was associated with more frequent moderate to vigorous physical activity and physical activity outside the school hours in children ($p < 0.05$). Other built environment dimensions, such as population density and street connectivity, were associated with lower physical activity. Multiple exposure models showed that some of the estimates were attenuated (eg. NVDI and street connectivity) and building density was associated with an increase of moderate-to-vigorous physical activity and extracurricular physical activity. These associations are consistent with findings from previous studies (Bringolf-Isler et al., 2014), which showed an inverse association of vegetation and building density with

sedentary behaviours and also obesity, and a positive association with physical activity in children and adolescents.

To conclude, these results suggest that several urban environment characteristics (more green space, building density, less population density and traffic load) may beneficially influence lifestyle behaviours in children and thus be protective for conditions (including overweight and obesity) related to these lifestyle behaviours (paper in preparation).

2.2. Built environment, physical activity, and obesity in Central European cohorts

Associations between characteristics of the built environment, physical activity levels and obesity have been analysed in two Central European cohorts in Slovenia and Croatia (unpublished). The Analysis of Children's Development in Slovenia 2013 (ACDSi 2013) is a cross-sectional, sentinel site study which recruited children from 11 primary schools in Slovenia. Of 4,236 children between 6 and 14 years initially invited to participate in the study, 3,476 children were consequently included in baseline assessment in 2013, i.e. 2% of children in this age bracket in Slovenia. The Croatian Physical Activity in Adolescence Longitudinal Study (CRO-PALS) is an observational, longitudinal study of 15-year old adolescents in the city of Zagreb (Croatia). Out of the 1408 students invited, 903 agreed to participate in the 2014 and 2015 baseline assessment.

Built environment exposures were assigned based on residential address and included:

- Walkability score – for a 1500 m network buffer from residential address and standardised across cohort participants
- Percentage of green and blue space surface as proportion of network buffer area
- Number of playgrounds within 1500 m network buffer

Outcomes were focused on physical activity levels and body composition of children, obtained at baseline. Information on moderate and vigorous physical activity per week (MVPA) was collected using a seven-day recall. Vigorous physical activity (VPA) was defined as any physical activities that increase heart and breathing rate and make the participant sweat. Moderate physical activity (MPA) was defined as 'lower intensity physical activities such as walking, biking to school, and recreational swimming. Participants recorded the number of hours and 15-minute increments they engaged in MVPA in the previous week. MVPA was expressed as minutes per day.

Body height and body mass were measured using a GPM 101 anthropometer and portable Tanita BWB-800P electronic scale, respectively. Skinfold, as an indicator of body fat, was measured using a Harpenden fat calliper applied to 7 (Slovenia) and 4 (Croatia) locations on the right side of the body. The sum of skinfold measurements was used for analysis.

We fitted regression models to data from Croatia and Slovenia with a random intercept effect on country and adjusting for age and sex of participants. Walkability and percentage of blue and green space were analysed as categories (tertiles), number of playgrounds as dichotomized variable (playground present/not present).

Table 2. Linear regression assessing the relationship between characteristics of the built environment with obesity measures and physical activity. Shown are effect estimates with 95% confidence intervals in brackets**.**

Built environment		Body mass index	Skinfold (sum)	Medium to vigorous physical activity
Walkability	Low	1.00	1.00	1.00
	Medium	-0.26 (-0.52 – 0.01)	-1.69 (-3.73 – 0.35)	5.72 (-2.67 – 14.11)
	High	-0.42 (-0.68 – -0.15)*	-2.75 (-4.80 - -0.71)*	-4.42 (-12.58 – 4.0)
% blue or green space	Low	1.00	1.00	1.00
	Medium	0.04 (-0.22 – 0.31)	-0.44 (-2.48 – 1.60)	5.28 (-3.14 – 13.71)
	High	0.41 (0.14 – 0.67)	2.74 (0.71 – 4.77)	2.38 (-6.00 – 10.74)
Playground		-0.51 (-0.75 - -0.28)*	-3.48 (-5.31- -1.66)*	-7.03 (-14.55 – 0.50)

* highlight statistically significant associations

Results suggest that both walkability of the residential neighbourhood as well as number of playgrounds within a neighbourhood are associated with a decrease in BMI and skinfold but not with physical activity levels. We did not find an effect with the percentage of blue and green spaces in these Eastern European cohorts: this is in contradiction with findings from other parts in Europe such as the HELIX cohorts described above, highlighting the fact that results are not necessarily transferable to other settings. The local context and built environment structure which differs across geographical regions needs to be taken into consideration when recommending policies (paper in preparation).

3. Internal exposome

3.1. Cord blood metabolic signatures predictive of childhood overweight and rapid growth

Metabolomics may identify biological pathways predisposing children to risk of overweight and obesity. In STOP we have investigated the cord blood metabolomic signatures of rapid growth in infancy and overweight in early childhood in four European birth cohorts (INMA, Rhea, Piccolipiù, Environage)(Handakas et al., 2021, https://www.nature.com/articles/s41366-021-00888-1). Untargeted liquid chromatography-mass spectrometry (LC-MS) metabolomic profiles were measured in cord blood from 399 newborns. Rapid growth in the first year

of life and overweight in childhood (mean age 5.4 years) were defined with reference to WHO growth charts. Metabolome-wide association scans (MWAS) for rapid growth and overweight on over 4500 metabolic features were performed using multiple adjusted logistic mixed effect models and controlling the false discovery rate (FDR) at 5%. Additionally, we performed a look-up analysis of 43 pre-annotated metabolites, previously associated with birthweight or rapid growth (Handakas et al, 2021).

In the MWAS analysis, we identified three and eight metabolites associated with rapid growth and overweight respectively, after FDR correction. Higher levels of cholestenone, a cholesterol derivative produced by microbial catabolism, was predictive of rapid growth ($p= 1.6 \times 10^{-3}$). Lower levels of the branched chain amino acid (BCAA) valine ($p= 8.6 \times 10^{-6}$) was predictive of overweight in childhood.

The area under the receiver operator curve for multivariate prediction models including metabolites identified in the MWAS analysis was 0.77 for rapid growth and 0.82 for overweight, compared to 0.69 and 0.69 respectively for models using traditional risk factors alone (sex, birthweight, ethnicity, maternal BMI, paternal BMI, gestational age, maternal weight gained during pregnancy, paternal education, maternal passive and active smoking status during pregnancy, parity and mode of delivery).

Among the 43 pre-annotated metabolites, seven and five metabolites were nominally associated ($P < 0.05$) with rapid growth and overweight respectively. The BCAA leucine remained associated (1.6×10^{-3}) with overweight after FDR correction. The metabolites identified here may assist in the identification of children at risk of developing obesity and improve understanding of mechanisms involved in postnatal growth. *Cholestenone and BCAAs* are suggestive of a role of the gut microbiome and nutrient signalling respectively in child growth trajectories.

3.2. Epigenetics: methylation-wide association study

The aim of our epigenetic analyses was to investigate the associations between blood DNA methylation at birth and rapid weight gain and childhood overweight in the 4 cohorts as above, plus GENERATION XXI (GXXI) and ALSPAC (Alfano et al, paper in preparation).

For a total of 1,919 children, cord blood methylation was measured using the Infinium HumanMethylation450 BeadChip for all the cohorts (ALSPAC, ENVIRONAGE, Rhea, INMA and PICCOLI+), except for GXXI that used Infinium MethylationEPIC BeadChip. Rapid growth in the first year of life and overweight in childhood (4-8 years) were defined with reference to WHO growth charts. Epigenome-wide association studies for rapid growth and overweight were performed using multiple adjusted logistic mixed effect models and then meta-analysed via fixed-effects meta-analysis weighted by the inverse of the variance. Models were adjusted for maternal education, pre-pregnancy BMI, tobacco smoke during the index pregnancy, maternal age at delivery, gestational age, parity, cell proportions, and on bead array row and bisulfite conversion batch as random effects. Results were considered significant if p-values adjusted for multiple testing by controlling the FDR were below 0.05. Additionally, we identified differentially methylated regions (DMRs).

In fully adjusted models we were not able to identify any individual CpGs associated with rapid growth and overweight, after p-value FDR correction. DMRs were associated with rapid growth (Table 3), including a

region located at the *PRDM16* gene which controls the development of brown adipocytes and that was previously found differentially methylated in children with obesity by (Fradin et al., 2017).

Table 3. Differentially methylated regions related to rapid growth.

Gene	Nr of CpGs	Chromosome	Start	Stop	P value	FDR
CLDN4	4	chr7	73241728	73242028	5.47E-09	4.92E-08
THEM5	4	chr1	1.52E+08	1.52E+08	7.01E-08	3.15E-07
GPX6	6	chr6	29454622	29454954	1.82E-07	5.14E-07
SPATA33	2	chr16	89734985	89735184	2.28E-07	5.14E-07
LINC01005	3	chr7	63386615	63386815	4.33E-07	7.79E-07
PRDM16	2	chr1	3240128	3240227	2.71E-06	4.06E-06

These 6 DMRs related to rapid growth were not able to improve prediction of childhood obesity in comparison with classical risk factors for obesity. Therefore, evidence on the role of epigenetics in childhood overweight or obesity is so far limited.

3.3. Systems biology: multiomic analysis and birthweight

Multiomic analysis, i.e. based on multiple measurements of changes in different compartments of molecules, has been published (Alfano et al., 2020). To investigate the systems biology of *birthweight*, we cross-sectionally integrated the methylome, the transcriptome, the metabolome and a set of inflammatory proteins measured in cord blood samples, collected from four birth-cohorts as above (ENVIRONAGE, Rhea, INMA and PICCOLI+). We focused on two sets of 68 metabolites and 903 CpGs previously related to birthweight and investigated the correlation structures existing between these two sets and all other omic features via bipartite Pearson correlations. The analysis revealed that the set of metabolome, proteome and methylome signatures of birthweight have seven signals in common, including three metabolites [PC(34:2), plasmalogen PC(36:4)/PC(O-36:5), and a compound with m/z of 781.0545], two CpGs (on the DHCR24 and SC4MOL gene), and two proteins (periostin and CCL22). Overall. the results of the omics integration indicated a central role of *cholesterol metabolism*; therefore, we explored the association of cholesterol levels in cord blood with birthweight in the ENVIRONAGE cohort (n = 1097), where cholesterol fractions were measured independently of metabolomics. We found that higher birthweight was associated with increased high-density lipoprotein cholesterol and that high-density lipoprotein cholesterol was lower in small versus large for gestational age newborns.

These data suggest that an integration of different omic-layers in addition to single omics studies is a useful approach to generate new hypotheses regarding biological mechanisms. Cholesterol metabolism in cord blood

may play a mechanistic role in birthweight, though it is not clear whether this is due to environmental or genetic influences.

4. Diet in children and molecular changes

4.1. Diet quality and insulin secretion in children in the HELIX consortium

Over the past decades, diets – also in children - have shifted towards the consumption of ultra-processed foods, characterized by higher energy density and lower nutritional quality. According to the NOVA food processing classification system, ultra-processed foods are defined as industrially-derived food and drink formulations of chemical compounds which include substances derived from foods but not used in culinary preparations, such as hydrogenated fats, and cosmetic additives. The NOVA classification has been instrumental in allowing the categorization of foods but is still imperfect and a better assessment of UPF intake requires good dietary data and more granularity than usually available.

In this study we aimed to examine the associations of Mediterranean diet adherence and ultra-processed food (UPF) consumption with urinary metabolites and serum C-peptide concentrations in children; C-peptide is a marker of endogenous insulin secretion. We studied 1147 children, (mean [SD] age 7.9 [range: 5.4-12.0] years), from the Human Early-Life Exposome (HELIX) project (consisting of six population-based birth cohorts in France, Greece, Lithuania, Norway, Spain, and the UK, same as above)(Fernandez et al, https://www.medrxiv.org/content/10.1101/2021.09.09.21263335v1). We assessed adherence to the Mediterranean diet using an a priori defined score (KIDMED). UPF intake was assessed based on the NOVA system and expressed as percent of total daily food intake. NOVA (4th release) is a food classification system (Monteiro et al., 2018) developed to assess the daily levels of UPF intake. Urine metabolomic profiles were measured using 1H nuclear magnetic resonance spectroscopy. We investigated their associations with C-peptide concentrations, which were assessed in child serum with the multiplex Luminex system, as well as Mediterranean diet and UPF association with metabolome profiles, by means of linear regression modelling adjusted for child body mass index and sociodemographic variables.

We found that a higher KIDMED score was associated with lower C-peptide levels. Compared to a low KIDMED score (<1), children with a moderate score (1-4) had a 28% lower C-peptide concentration (percent change: -27.7, 95% CI: -49.6 to 3.9) and those with a high score (>4) had a 39% lower C-peptide concentration (percent change: -39.0, 95% CI: -60.6 to -5.7) (P-trend 293 =0.03).

An opposite association was observed for UPF intake. Compared to children at the lowest quartile of UPF intake (<18% of total daily food intake), children at the second quartile (18-<23% of total daily food intake) had a 24% higher C-peptide concentration (percent change: 24.3, 95% CI: -6.4 to 65.2), those at the third quartile (23-<29% of total daily food intake) had a 39% higher concentration (percent change: 38.5, 95% CI: 3.8 to 84.9), and those at the fourth quartile (≥29% of total daily food intake) had a 46% higher concentration (percent change: 46.0, 95% CI: 8.1 to 97.3) (P-trend=0.01).

C-peptide concentrations in children is a marker of endogenous insulin secretion; lower levels are considered to be associated with higher risk of diabetes (Fernandez et al, https://www.medrxiv.org/content/10.1101/2021.09.09.21263335v1).

Like in the studies summarized above and below, this paper also observed a decrease in BCAA in association with UPF.

4.2. Ultra-processed food and metabolomic profile in the ALSPAC cohort

In this study, we examined the associations of ultra-processed food (UPF) consumption with metabolic traits. Initially, the ALSPAC study recruited 14,541 women living in Avon (UK), with an expected delivery date between 1 April 1991 and 31 December 1992. Participants have been followed up with regular questionnaires and clinical measures, providing lifestyle, behavioural and biological data. The ALSPAC study website contains details of all the data that is available through a fully searchable data dictionary search tool (http:// www.bris.ac.uk/alspac/researchers/data-access/data-dictionary/).

For dietary assessment we used NOVA as above. Metabolic profiling was carried out using ^{1}H nuclear magnetic resonance spectroscopy on fasting plasma samples from the ALSPAC cohort. This molecular signature of systemic metabolism consists of 232 metabolic traits. The platform provided quantification of 14 lipoprotein subclasses (particle concentration, lipid concentrations and composition), fatty acids and fatty acid composition, ketone bodies, amino acids, gluconeogenesis-related metabolic traits and glycolysis and gluconeogenesis-related metabolites (Kujala et al., 2013; Soininen et al., 2009). We investigated the association between the metabolomic or epigenetic data and UPF consumption using multiple linear regression at 7 years of age. Additionally, we examined the longitudinal association of UPF assessed at 13 years and metabolomics assessed at 15 years. All the models were adjusted for child age, sex and BMI, maternal age, maternal education level, pre-pregnancy BMI, family income, average time spent on TV, presence of a smoker at home, and physical activity , as fixed effects.

In the metabolomic analysis to account for multiple testing a Bonferroni correction for Effective Number of Tests (based on principal components explaining 95% variance) was applied ($p < 0.005$). For a total of 4357 children, the analysis showed that a diet with a higher proportion of UPF was negatively associated with docosahecaenoic acid and omega-3 fatty acids (generally considered biomarkers of fish consumption), tyrosine and BCAAs leucine, valine and isoleucine. Numerous intervention studies and animal studies have shown that higher intake of BCAAs has beneficial signalling effects, with positive impact on parameters including body composition, glycemia and satiety (Lynch & Adams, 2014; Pallares-Méndez, Aguilar-Salinas, Cruz-Bautista, & del Bosque-Plata, 2016). Proposed mechanisms for these positive effects include a direct action on hypothalamic and brainstem processes involved in satiety (Lynch & Adams, 2014). Lower blood BCAAs levels could therefore influence later propensity for overweight through intermediate processes such as control of food intake. Citrate, glutamine and creatinine were positively associated with UPF consumption. Additionally, negative associations were found for various lipoprotein subclasses such as very large density lipoproteins (VLDL), intermediate density lipoproteins (IDL), large and very large high density lipoproteins (HDL), and small low-density lipoproteins (LDL) (Handakas et al., submitted 2021)

To understand the role of plasma metabolic profiles in adiposity trajectories, linear growth curve models were used to investigate the longitudinal associations between baseline quartiles of metabolic features at 7 years and fat mass measured at ages of 9, 12, 15 and 17 years of age (n= 4357 individuals, N= 12987 measurements). Increases in fat mass per year were significantly greater for children with the highest metabolite quartiles for glutamine, citrate, MUFA, MUFA as ratio to total fatty acids, LDL triglycerides, IDL, small, medium, and large LDL and total cholesterol. Increases in fat mass per year were significantly lower for children with the highest metabolite quartiles for isoleucine, leucine, phenylalanine, and tyrosine. Evidence for a dose response across quartiles was observed for isoleucine, leucine, phenylalanine, tyrosine, citrate, MUFA as ratio to total fatty acids, IDL and medium LDL. Citrate is the most widely used additive in the food industry, as it is a very efficient food flavouring agent and preservative (Evans, de Challemaison, & Cox, 2010). The growth curve analysis showed that higher levels of both citrate and glutamine at 7 years were associated with greater fat mass accumulation.

Overall, this analysis revealed that higher level consumption of UPF during childhood is associated with altered metabolomic profiles. Higher levels of citrate, glutamine and MUFA and lower levels of BCAAs and AAAs may contribute to the association between UPF and fat mass accumulation in children. Our findings shed light on metabolic effects of nutrient-poor diets and provide potential mechanisms underlying the harmful effects of UPF. However, these results need replication and have limitations in relation to the NOVA classification of UPF and the underlying limited granularity of dietary information.

5. Connecting the external with the internal exposome

An assumption of exposome research is that internal changes (metabolomic or molecular) help understand the relationships between external and behavioural exposures and outcomes such as rapid growth, overweight or obesity. According to the “meet-in-the-middle” model the aim is to strengthen causality by finding intermediate markers that are associated with both exposures and outcomes, thus lending biological credibility to statistical associations (Chadeau-Hyam et al., 2011).

This has been attempted in a recent HELIX study (Maitre et al, 2021, https://www.medrxiv.org/content/10.1101/2021.05.04.21256605v1.full). This analysis has not been conducted within STOP but is based on the same cohorts analyzed above and therefore is a relevant complement to the present deliverable. In a multi-centre cohort of 1,301 mother-child pairs, they associated individual exposomes consisting of >100 chemical, physical and lifestyle exposures assessed in pregnancy and childhood, with multi-omics profiles (methylome, transcriptome, metabolome and proteins) in childhood. They identified 1,170 associations, 249 in pregnancy and 921 in childhood, which revealed potential biological responses and sources of exposure. The methylome best captures the persistent influence of pregnancy exposures, including maternal smoking; while childhood exposures were associated with features from all omics layers, revealing novel signatures for indoor air quality, essential trace elements, endocrine disruptors and weather conditions. In particular, we found several methylation or omic associations for *indoor air quality during childhood*, in contrast to the few associations found for outdoor air pollution. Indoor levels of PM2.5 absorbance, a marker of black/elemental carbon originating from combustion, were associated with methylation of 9 CpGs, including

two in common with tobacco exposure, and with decreased levels of serum branched amino acids (BCAA: Ile, Leu, and Val), acylcarnitine C4 (butyrylcarnitine) and two sphingolipids. Lower BCAA and acylcarnitines were detected in young obese participants exposed to near-roadway air pollution (Chen et al., 2019). Associations between dysregulated metabolism of BCAAs and acylcarnitines with obesity and insulin resistance have been widely observed in animal and adult human studies (Newgard, 2017).

We propose that altered BCAA and acylcarnitine metabolism may be an important biomarker to study further in relation to indoor air pollution and subsequent development of cardio-metabolic disease in later life. An association between indoor air pollution and increased child BMI was previously reported in the HELIX study, independently of correlated exposures such as second-hand smoke and lower social class status (Vrijheid et al., 2020), nlso in the systematic review by Malacarne et al summarized above. Other associations that we do not consider here were with chemical exposures and their internal markers.

These observations confirm that intermediate markers can be found linking characteristics of the living environment (in particular indoor exposures) with metabolic or epigenetic changes, that in turn have been associate with childhood obesity.

6. Social disparities and obesity in children in the Lifepath consortium

We are currently analyzing the relationships between socio-economic position and obesity in STOP. Here we summarize evidence from a previous H2020 project. *Lifepath* is a H2020-funded consortium of population investigations aiming to address the impact of social disparities on health and poor ageing. *Lifepath* includes several children cohorts in Europe and results of its research indicate that socioeconomic circumstances cast a long shadow “from the womb to the tomb”. Disadvantaged socio-economic position (SEP) at birth, in early years and childhood negatively influences health in adulthood and old age. We investigated the relationship between SEP (represented by maternal education) and obesity by analysing data on body mass index (BMI) from 41,399 children in three prospective cohort studies: Generation XX1 (G21 – Portugal), Growing Up in Ireland (GUI – Ireland), and the Millennium Cohort Study (MCS – UK). Infants, children and adolescents from disadvantaged SEP backgrounds were more likely to be overweight, with disadvantaged children having a higher body mass index from as young as age three (McCrory et al, 2019). This result is similar to previous estimates indicating that the social gradient in BMI emerges as early as nine months of age (Roy et al, 2016), or three or four years depending on the studies (Layte, 2014; Howe et al, 2011). Previous research indicates that around half of the social class differential in obesity risk in early life reflects patterns of breastfeeding and early weaning onto solid foods (Layte et al, 2014). Obesity and overweight are associated with increased risk of type 2 diabetes, hypertension and cardiovascular disease (Sahoo et al, 2015).

Also the intake of ultraprocessed food has been found strongly associated with SEP in the ALSPAC cohort, in a paper that also found an association of UPF with childhood adiposity trajectories (Chang et al, 2021). As a whole, this evidence has important implications for health and social policy.

7. Policy suggestions

In our research in the STOP consortium, we have primarily focused on maternal factors in the prenatal analyses, mainly due to data availability, but the role of paternal factors is increasingly recognised in developmental research. Withstanding these limitations, we propose the following highlights and suggestions.

Highlights of the findings:

Geographic studies based on georeferencing of individuals included in childhood cohorts suggest that contextual variables such as green spaces or walkability may be related to physical activity, overweight and obesity. Still uncertain is the role of different variables related to food availability and intake from spatial studies, and their association with overweight and obesity.

Our literature reviews on metabolomics and epigenetics suggest that the evidence is still immature and too heterogeneous to draw strong inferences, though metabolomics in cord blood points to a relationship between cholestenone and childhood obesity/overweight. It is unclear if these changes are related to maternal behaviours and exposures or have a genetic origin.

Our biomarker-based studies strongly suggest (in several ways, including mediation analysis) Branched-Chain Amino Acids (BCAAs) as mediators of risk factors for childhood overweight/obesity. This is consistent with intervention studies and animal studies showing that higher intake of BCAAs has positive impact on parameters including body composition, glycemia and satiety. Blood BCAAs levels could influence later propensity for overweight through causal processes such as control of food intake contributing to effects of dietary patterns on weight gain. However, mechanisms still need clarification.

Concerning food, of particular concern is the trend of increasing intake of ultra-processed food, including among children. Research from the ALSPAC cohort has shown a relationship between UPF intake and trajectories in childhood adiposity (Figure 1). In ALSPAC (Handakas et al, submitted), UPF was related to lower blood levels of BCAAs providing a potential mechanism underlying control of food intake and obesity risk.

According to the NOVA food processing classification system, ultra-processed foods are defined as industrially-derived food and drink formulations of chemical compounds which include substances derived from foods but not used in culinary preparations, such as hydrogenated fats, and cosmetic additives. UPF are energy dense but nutritionally poor, and have been involved in higher risk of disease in adults (Seferidi et al, 2020). The NOVA classification needs refinement and its use is strongly related to the quality and granularity of dietary assessments. Several mechanisms have been proposed to explain the impact of UPF on obesity and overweight in adults, including changes in nutrient intake, changes in appetite (possibly mediated by gut hormones) or the role of additives. In particular, experimental studies on food consumption indicate that ultra-processed foods have low satiety potential and induce high glycaemic responses (Fardet, 2016; Monteiro et al., 2019).

Further research is needed on the role of UPF. Different types of UPF should be analyzed, and their mechanisms of action should be clarified. This would lead to more specific reformulations and dietary recommendations. Opportunities for such research are offered by the RCT planned in STOP WP8.

Socio-economic position is a powerful driver of overweight and obesity in children, probably through several pathways and mechanisms, including lower opportunities for physical activity (also related to the built environment) and poorer dietary habits (including lower intake of BCAA and higher intake of UPF).

As guidelines for preventive action, our research suggests a few interventions that are supported by evidence and should be implemented:

1. Limit intake of ultra-processed food in infancy, by limiting the proportion of calories they represent in diet
2. Diversify children diets, with emphasis on fresh food (and in particular rich in BCAA)
3. Create opportunities for physical activity, including urban planning (safe areas reserved to children, green spaces, blue spaces, biking lanes) and promotion of sport activities at school.

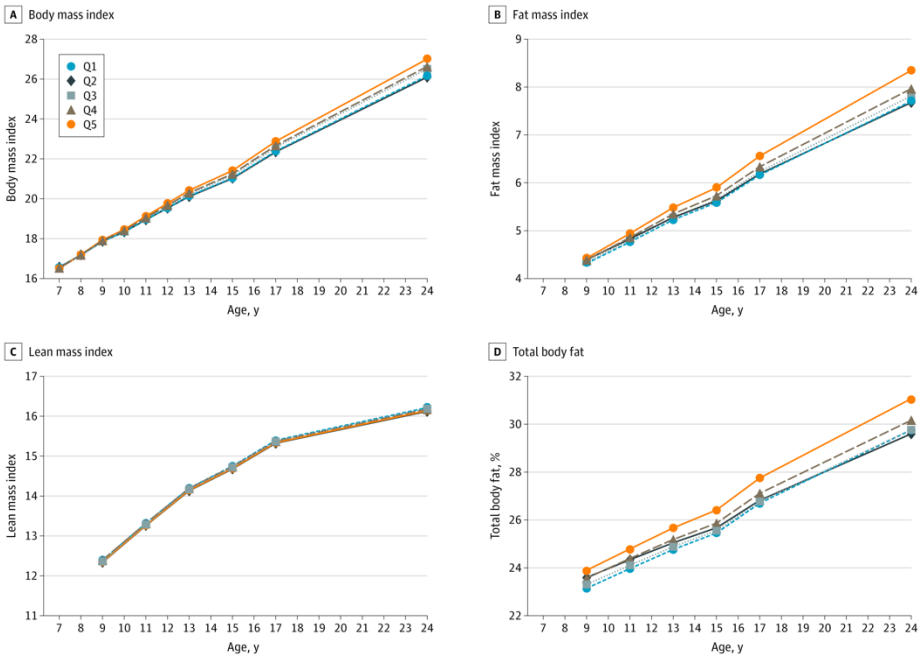

Figure 1. Trajectories of Primary Outcomes by Baseline Quintile of Ultraprocessed Food (UPF) Consumption Data are from 9025 children who participated in the Avon Longitudinal Study of Parents and Children. Percentage of daily food intake contributed by UPFs at baseline was categorized into quintiles (Q1-Q5, lowest to highest quintile of UPF consumption)(Chang et al, 2021).

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