Science and Technology in childhood Obesity Policy

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 774548.

EC Framework Programme for Research and Innovation

Horizon 2020

H2020-SFS-2017-2-RIA-774548-STOP:

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Start date of project: 1st June 2018 Duration: 48 months

D3.4: Report on Gut Hormones Measurement

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Version: Draft 2

Preparation date: 30/03/21

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
BMI	Body mass index
PYY	Peptide YY
GLP-1	Glucagon-like peptide 1
GI	Gastrointestinal
NW	Healthy weight
OB	Overweight/obese
NPY	Neuropeptide Y
AgRP	Agouti-related protein
POMC	Proopiomelanocortin
SNP	Single nucleotide polymorphism

Science and Technology in childhood Obesity Policy

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1. Introduction

The aim of the deliverable 3.4 in STOP (Science & Technology in childhood Obesity Policy) is to understand how dietary components and profiles influence appetite regulation and weight gain through gut hormone release in children. This will enable mechanistically driven food policy in the prevention and management of obesity. For this, data from Generation 21, Inma, Environage and Rhea cohorts have been analysed to measure gut hormones levels (GLP-1, PYY and ghrelin). The aspiration is to be able to identify: 1) the effects of overweight/obesity on gut hormones; 2) the effects of gut hormones on weight gain; 3) the effects of diet on gut hormones; and 4) the role of gut hormones as mediator between diet and weight gain.

1.1 Obesity in Children

Worldwide, childhood obesity incidences have been increasing at an alarming rate, with higher prevalence associated with the westernised culture [1]. The pathogenesis of obesity involves a complex interplay of genetic susceptibility, environmental, and lifestyle factors (WHO, 2020). One of the suggested mechanisms leading to obesity involves a mismatch in the energy homeostasis dynamics. When energy intake exceeds energy expenditure, a state of positive energy balance occurs and the consequence is an increase in body weight. There has been great interest in understanding this complex disease particularly due to the multitude of metabolic complications associated with obesity such as insulin resistance, cardiovascular disease and non-alcoholic fatty liver disease (Horner and Lee, 2015). Children that are diagnosed with obesity are likely to remain obese as adults, furthering their risk of developing these comorbidities (Liberali et al., 2020)

1.2 Gut Hormones

Children that are diagnosed with obesity are primarily recommended lifestyle interventions, including exercise and dietary changes as the primary form of treatment to reduce their body weight (Salam et al., 2020) Appetite regulation is one of the key targets in the dietary treatment of obesity (Butland et al., 2008). Appetite is regulated by a multidisciplinary system consisting of integration in the brain of peripheral signals from the gastrointestinal tract, adipose tissue and other organs (Ahima and Antwi, 2008).

Peripheral signals that regulate appetite and energy homeostasis largely derive from the gastrointestinal (GI) tract (Murphy and Bloom, 2006). These signals include the anorexigenic hormones peptide YY (PYY) and glucagon-like peptide 1 (GLP-1) and the orexigenic hormone ghrelin. Anorexigenic hormones circulate at moderately low levels before meal ingestion and at higher levels after a meal, while ghrelin circulates at high levels before meal consumption and decreases sharply after meal ingestion (Tschop et al., 2001). Together, these hormones and others regulate feelings of appetite and food intake thus, influencing body weight.

During childhood PYY, GLP-1 and ghrelin are critical in meeting energy demands throughout development. The body will demand more energy to grow and carry out physiological functions at

specific periods of lifespan - neonatal, infancy, puberty (Prodam et al., 2014). Carefully altering the expression of these hormones allows for these increased energy demands to be met without inducing a positive energy balance that would lead to weight gain. Dysregulation of such hormones has been implicated in the pathogenesis of obesity (Horner and Lee, 2015). Understanding the differences in gastrointestinal hormone secretion between healthy weight and obese children across different developmental stages could help to identify underlying mechanisms involved in weight gain and highlight potential prevention and treatment strategies.

1.3 Systematic Review: Gut Hormones Levels from Birth to Adolescence

Gastrointestinal hormones play an important role in energy homeostasis regulation by responding to nutritional intake in the gut and signalling to the brain to control appetite. The secretion of these hormones responds according to the energy demands required at different stages of development (neonatal, infancy, adolescence, adulthood). Disruption to this secretion has been implicated in the pathogenesis of obesity. Understanding the differences in gastrointestinal hormone secretion between healthy weight and obese children across different developmental stages can help to identify underlying mechanisms involved in weight gain and highlight potential treatments.

A systematic review was conducted to compare ghrelin, PYY and GLP-1 levels in fasting state or in response to feeding in healthy weight and obese children, from birth to adolescence. Where studies reported on appetite score, this was also discussed. Systematic searches were conducted in PubMed, Web of Science and Cochrane library. References from relevant review papers published in the last five years were also hand searched. The eligibility criteria for the studies were as follow: a) study must have measured at least ghrelin, or GLP-1 or PYY; b) must have been conducted in humans, neonates to adolescents, aged (0-18); c) must have included healthy weight and/or overweight or obese participants; d) studies involving metabolic disorders or eating disorders were excluded; e) papers that did not define BMI of participants or groups were also excluded f) must have had a full text with primary data. A total of 732 papers were retrieved of which 67 met the inclusion criteria. Obese children compared with healthy weight children seem to have lower fasting ghrelin levels and attenuated ghrelin responses to caloric intake across all age ranges. This pattern is not observed for PYY; fasting and postprandial PYY levels appear not to differ in obese and healthy weight children across all ages studied. There is considerable evidence to suggest that ghrelin and PYY levels reduced with maturation, independent of the body mass index (BMI). There was little to no consensus in the literature for GLP-1 levels in obese and healthy weight children. Many papers also reported there to be no differences in sex or score of appetite for these three hormones.

This review highlighted a solid trend for ghrelin and PYY levels in fasting and postprandial gastrointestinal hormone levels in healthy weight and obese children which was observed across different stages of development. It also commented on the limited number of publications on GLP-1 levels in children, an area which needs further investigation to provide a more comprehensive overview of how these gastrointestinal hormones work together to regulate body weight.

2. Methods

2.1 Gut Hormones Measurement

2.1.1 Peptide YY (PYY) and Glucagon-like-peptide 1 (GLP-1)

PYY and GLP-1 immunoreactivity were measured with specific and sensitive in-house radioimmuno-assays, as previously described (Allen et al. 1984, Kreymann et al. 1987). In brief, for PYY, the assay uses a polyclonal antibody which detects both biologically active forms, $PYY_{3–36}$ and $PYY_{1–36}$. This antibody does not cross-react with other known gastrointestinal hormones. The assay involves competition for antibody binding sites between a fixed amount of radiolabelled peptide and the amount of peptide in the sample or standard. A standard curve was established from which the concentrations of samples were calculated. Iodine-125–labelled PYY was prepared by the Iodogen method and purified by high-pressure liquid chromatography. The assay was performed in 0.06 M phosphate buffer, pH 7.3, containing 0.3 percent bovine serum albumin. Samples were added in duplicate. The assay incubated for three days at 4°C before the separation of free and antibody-bound label by charcoal absorption. The fractions were then measured using a Gamma Counter (Multi Crystal LB 2111, Berthold, Germany). GLP-1 was measured in duplicate using a similar technique as described for the PYY assay. The GLP-1 assay uses a polyclonal antibody which recognises the GLP-1 1-36 amide, GLP-1 7-36 amide and GLP-1 9-36 amide forms of the hormone. The hormones for each cohort were measured in an individual assay, totalizing four different assays. Intra and inter-assay coefficients of variation (CV) for PYY and GLP-1 assays were <10%.

2.1.2 Ghrelin

Total ghrelin levels were determined by a two-site sandwich enzyme immunoassay (EMD Millipore Corporation, Missouri, USA) using a plate reader (ELx808 Absorbance Microplate Reader, Winooski, USA) according to the manufacturer instructions. Intra and inter-assay CV for the total ghrelin assays were <10%.

2.2 Statistical Analysis

All the data was analysed with the assistance of the Statistical Package for Social Sciences (SPSS) version 25.0 (SPSS Inc., Chicago, Ill., USA) and GraphPad-Prism 9 (GraphPad Software, La Jolla California, USA). Normality of data was first assessed using the Shapiro–Wilk test. To compare the differences between fasting levels of the gut hormones in the two BMI categories – healthy weight and overweight/obese - at age 4 or 10 years old, the Mann-Whitney test was used. Wilcoxon's rank-sum test was used to assess differences between fasting values of GLP-1, PYY and ghrelin at age 4 compared to age 10. To determine differences in the delta change between the 3 groups (NWxNW, NWxOB and OBxOB) for GLP-1 and PYY levels, the Kruskal-Wallis test was conducted. A mixed

model ANCOVA was conducted, using the cohorts G21 and Inma as covariates, to assess the mixed effect of time and time x groups for the 3 groups (NWxNW, NWxOB and OBxOB) on GLP-1 and PYY levels. For the purpose of hypothesis testing, the 95% level of confidence was predetermined as the minimum criterion to denote a statistical difference ($p<0.05$). Data are expressed as mean ± standard error of the mean (SEM).

3. Results and Discussion

3.1 Characteristics of the Study Participants

Characteristics of the study population are described on Table 1. As expected, anthropometric data differed between healthy weight and overweight/obese groups and between 4 and 9-10 years old groups. Gut hormones data will be discussed in the following sections.

3.2 Effects of Overweight and Obesity on Gut Hormones

Data from four cohorts - Generation 21, Inma, Environage and Rhea - were used to obtain the results of GLP-1 and PYY described in this section. Data from Generation 21 was used to describe the total ghrelin results.

3.2.1 GLP-1

Lower fasting levels of GLP-1 were observed in overweight/obese compared to healthy weight children at 9-10 years old (39.59 ± 1.40 vs 47.09 ± 1.35 pmol/L, respectively; $p<0.001$). On the other hand, these differences were not seen in younger children (39.58 ± 1.84 vs 42.95±1.18 pmol/L, overweight/obese vs healthy weight; $p>0.05$) at 4 years old (Figure 1). There is no consensus in the literature with regards to GLP-1 levels in children, but most of the papers show no difference in GLP-1 levels in obese and healthy weight children (Giannini et al., 2016; Roth at al. 2019; Vien et al., 2017; Galderisi et al., 2019; Nguo et al., 2019; Beglinger et al., 2014; Manell et al., 2016). However, there are some studies reporting conflicting results. Tree studies (Lomenick et al. 2009a; Tomasik et al., 2002; 2009) have reported obese children to have significantly lower fasting levels of GLP-1 compared with healthy weight children, whereas the study of Lomenick et al. (2009a) have reported obese children to have significantly higher fasting levels of GLP-1 compared with healthy weight children.

Some factors that could be contributing for the disruption of GLP-1 levels in obesity are described in the literature and include an altered postprandial response to nutrients, abnormal circulating levels of glucose and insulin and also impaired signalling to the central nervous system and vagus nerve. Many studies in the literature have shown a blunted GLP-1 response to nutrients in overweight and obese compared with normal-weight children and adolescents (Tomasik et al. 2004; Stock et al. 2005; Chanoine et al. 2008; Misra et al. 2009; Mittelman et al. 2010; Roth et al. 2010; Beglinger et al. 2014). The aberrant postprandial gut hormone responses in obesity may be the result of an altered number and responsiveness of gut hormones secreting intestinal enteroendocrine cells. The altered gut microbiota composition in obesity may also mediate effects on gut hormone secretion through interactions with intestinal enteroendocrine cells (Koliak et al., 2020). GLP-1 also acts as a potent incretin hormone, promoting glucose-dependent insulin secretion. Abnormalities in glucose homeostasis and altered insulin secretion and sensitivity, as seen in obese individuals, could also be contributing to the disturbed levels of GLP-1 (Koliak et al., 2020). At the central nervous system,

there is evidence that altered signalling to NPY/AgRP and POMC Axis could occur (Geloneze et al., 2017). Impaired plasticity of the vagus nerve could also be occurring and, in consequence, leading to blunted effects of gut hormones on vagal nerve fibres (Koliak et al., 2020). Naturally occurring single nucleotide polymorphisms (SNPs) at the GLP-1R have been characterized in the literature, however there is limited knowledge on the physiological effects of these SNPs in the organism with some studies describing impacts on cardiovascular and metabolic functions. The presence of SNPs may also be linked to the rate of onset of some diseases or effectiveness of receptor targeted treatments (Koole et al., 2011; Ma et al., 2018). In adults this is a field of study that still needs further understanding. In children, the role and effect of GLP-1 receptor polymorphisms is less understood than in adults and its potential effect on GLP-1 levels in the youth cannot be discarded.

The evidence of lower fasting GLP-1 levels in the overweight/obese compared to lean children at 9-10 years old indicates that GLP-1 is dysregulated in overweight and obese youth. The lack of studies on the levels of GLP-1 in children and adolescents makes it difficult to compare the results and also highlights the need of more studies in the field.

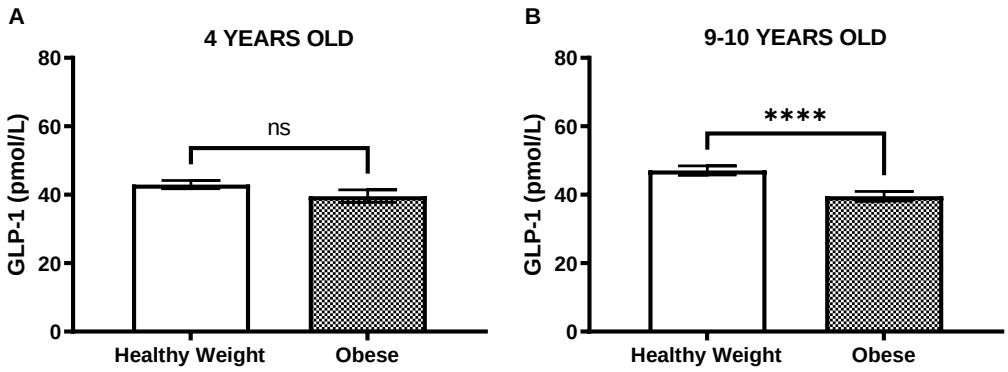

*Figure 1. GLP-1 levels in healthy weight vs. overweight/obese children at 4 years old (A) and at 9-10 years old (B). Values are in mean ± SEM. Mann-Whitney test, **** p<0.0001; ns = non-significant (p>0.05).*

3.2.2 PYY

No differences were observed in fasting PYY levels in overweight/obese compared to healthy weight children at either 4 years old (23.62 ± 2.17 vs 21.83 ± 0.95 pmol/L, respectively; p>0.05) or at 9-10 years old (17.83 ± 2.13 vs 16.28 ± 0.82 pmol/L, respectively; p>0.05). See Figure 2. Fasting PYY levels have been reported to be similar between overweight/obese and normal-weight youth in many studies (Roth et al., 2010; 2019; Lomenick et al., 2008; 2009; Mittleman et al., 2010; Van Name et al., 2015; Nguo et al., 2019; Stock et al., 2005; Misra et al., 2009; Sysko et al., 2013), but not all. Vien et al. (2017), reported obese children to have significantly lower fasting PYY levels compared to healthy weight children. Patel et al. (2014), concluded otherwise, describing that obese children had significantly higher fasting levels of PYY compared to healthy weight children. The results

presented in the current study are in accordance with the majority of the evidence available in the literature. Therefore, PYY doesn't seem to be affect by BMI, independent of the age.

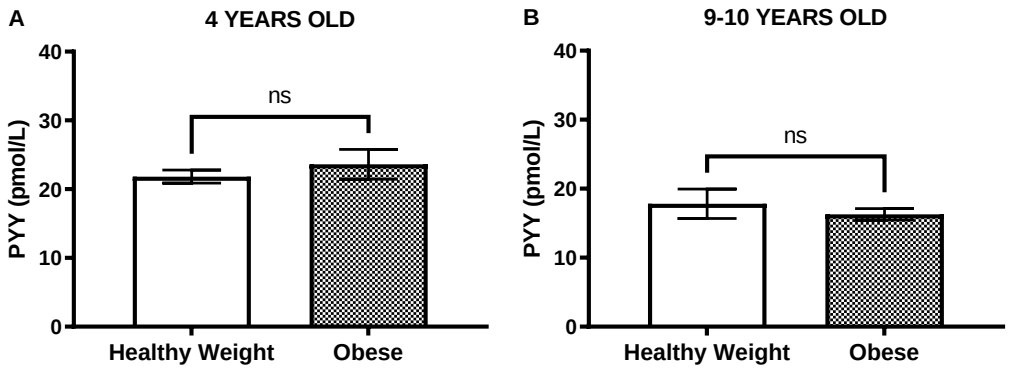

Figure 2. PYY levels in healthy weight vs. overweight/obese children at 4 years old (A) and at 9-10 years old (B). Values are in mean ± SEM. Mann-Whitney test, ns = non-significant (p>0.05).

3.2.3 Total Ghrelin

Lower fasting levels of total ghrelin were observed in overweight/obese compared with healthy weight children at either 4 years old (568.40 ± 38.15 vs 719.00 ± 35.54 pg/mL, respectively; $p<0.05$) and at 10 years old (398.40 ± 20.59 vs 510.60 ± 20.59 pg/mL, respectively; $p<0.01$). See Figure 3. The literature has been consistently reporting lower fasting ghrelin levels in overweight/obese compared with normal weight children and adolescents. Two studies [13], [14] reported fasting total ghrelin levels in healthy weight and obese children aged 4 years old, with both showing levels to be significantly lower in obese than in healthy weight children. In older children, aged 6-12 years old, studies have reported one or more molecular forms of ghrelin to be lower in obese than in healthy weight (Aly et al., 2020; Pacifico et al., 2009; Bellone et al., 2002; Fisher et al., 2007; Zhu et al., 2010; Vivenza et al., 2004; Razzaghy-Azar et al., 2016; Bacha et al., 2005; Baldelli et al., 2006; Podram et al., 2011; Roth et al., 2019; Lomenick et al., 2009b; Soriano-Guillén et al., 2004). However, these findings are not unanimous as there are also some studies reporting no differences in fasting total ghrelin levels in children at this age range (Roth et al., 2010; Bascietto et al., 2012; Lomenick et al., 2008; Vien et al., 2017; Gil-Campos et al., 2010; Mittelman et al., 2010).

Similar to GLP-1, the postprandial response of ghrelin also seems to be disrupted in the obese. The suppression of total ghrelin is lower in overweight/obese compared with normal-weight youth in response to glucose or meal ingestion - for a fixed load test meals (Tschop et al., 2001; Bacha and Arslanian 2005; Stock et al. 2005; Bacha and Arslanian 2006; Baldelli et al. 2006; Lomenick et al. 2008; Mittelman et al. 2010; Patel et al. 2014; Sysko et al. 2013; Beglinger et al. 2014). Ghrelin is negatively correlated with factors which are raised in obesity namely total adiposity (assessed by BMI, percent body fat and fat mass) and insulin levels. There is evidence that ghrelin suppression is positively correlated with insulin sensitivity and a blunted ghrelin suppression could be a manifestation of the insulin resistance in obesity. In addition, abnormal glucose homeostasis inversely correlates with, and was found to be an independent determinant of, plasma ghrelin levels in obese children and adolescents. At the central nervous system, there is evidence that diet inducing

obesity causes ghrelin resistance by reducing the NPY/AgRP responsiveness to ghrelin and suppressing the neuroendocrine ghrelin axis in an attempt to limit further food intake (Briggs et al. 2010; Zhu et al., 2010, Scerif et al., 2011).

Lower fasting ghrelin levels were shown in this study in overweight and obese compared with normal weight children, independent of the age - at 4 and at 10 years old. These results are in agreement with most of the data available in the literature. Taken together, this evidence suggests that ghrelin levels are dysregulated in overweight/obese children.

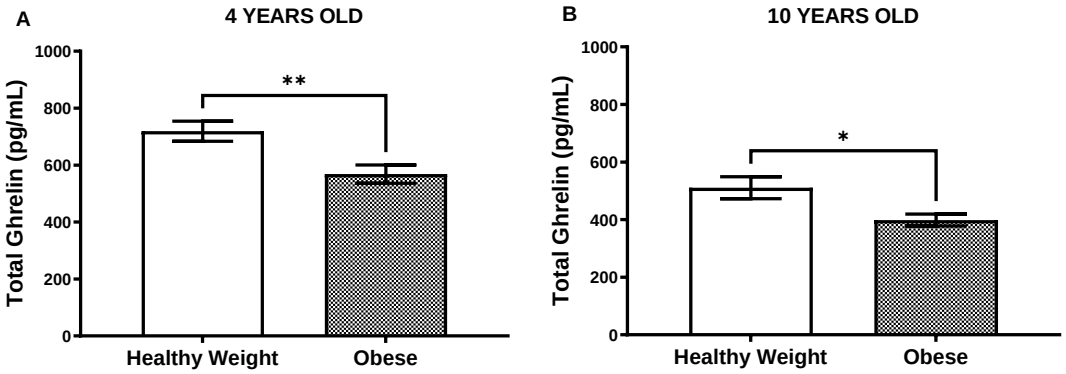

*Figure 3. Total ghrelin levels in healthy weight vs. overweight/obese children at 4 years old (A) and at 10 years old (B). Values are in mean ± SEM. Mann-Whitney test, **p<0.01; *p<0.05.*

3.3 Effects of Gut Hormones on Weight Gain

Data from two cohorts, Generation 21 and Inma, were used to compare the GLP-1 and PYY results at 4 and 9-10 years old. Data from Generation 21 at 4 and 10 years old was used to describe the ghrelin results. The data was divided in three groups. In the first group, the data from healthy weight children at both, 4 and 9-10 years old (NWxNW group) was included. The second group, was formed by the data from children that had a healthy weight at 4 but became overweight/obese at 9-10 years old (NWxOB group). In the last group, the data from overweight/obese children at both, 4 and 9-10 years old (OBxOB group) was included.

3.3.1 GLP-1

A mixed effect ANCOVA has shown an effect of time x group ($p<0.005$) for GLP-1 levels when comparing the 3 groups at ages 4 vs 9-10 years old (Figure 4A). The delta change analyses for GLP-1 revealed that groups NWxOB and OBxOB displayed reduced levels of GLP-1 when compared to the NWxNW group ($p<0.005$) - Figure 4B. When each group was analysed separately, the NWxOB (Figure 5B) and OBxOB (Figure 5C) groups showed that the levels of GLP-1 were reduced at 9-10

compared to 4 years old. The GLP-1 levels in the NWxNW group did not differ when comparing the two ages (Figure 5A).

The same factors discussed in the previous GLP-1 results section could also be explaining the disrupted levels of GLP-1 observed in children with excess of weight or excessive weight gain with age, including an altered postprandial response to nutrients, abnormal circulating levels of glucose and insulin and also impaired signalling to the central nervous system and vagus nerve (Koliak et al., 2020). One study in the literature have looked at the effect of maturation on GLP-1 levels. Schwartz et al. (2015), reported that fasting GLP-1 levels did not differ during maturation (pre-early vs mid-late puberty) in obese males.

The lack of studies in children looking at the effect of weight gain with age makes it difficult to compare the results. However, the current results suggest that weight gain may have an impact on GLP-1, reducing the hormone levels with age if the children were or became overweight/obese.

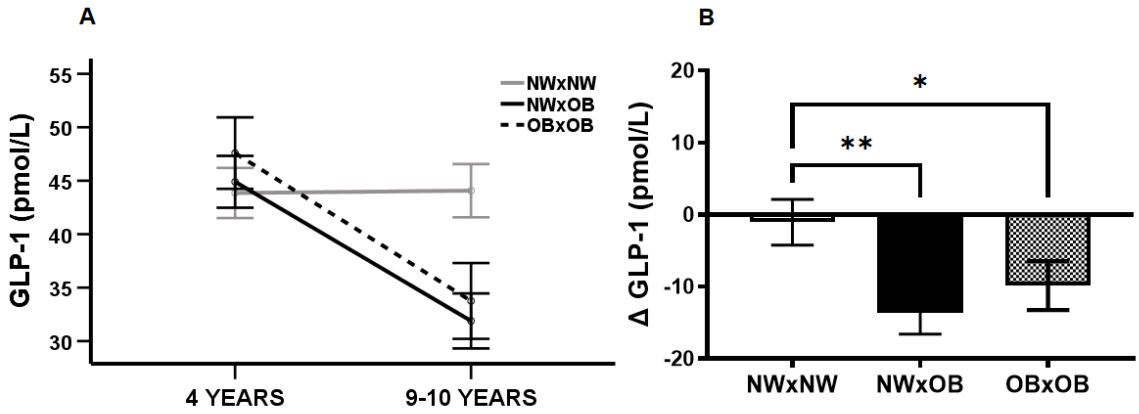

*Figure 4. ANCOVA comparison of the GLP-1 levels at 4 and 9-10 years old in the tree groups - NWxNW, NWxOB and OBxOB (A); GLP-1 delta change comparison between the tree groups (B). Values are in mean ± SEM. ANCOVA covariates appearing in the model are evaluated at the following values: G21=1 and Inma=2 = 1.34, effect of time p>0.05; effect of time x group p<0.005. Kruskal-Wallis test, **p<0.005. NW = healthy weight children; OB = overweight/ obese children.*

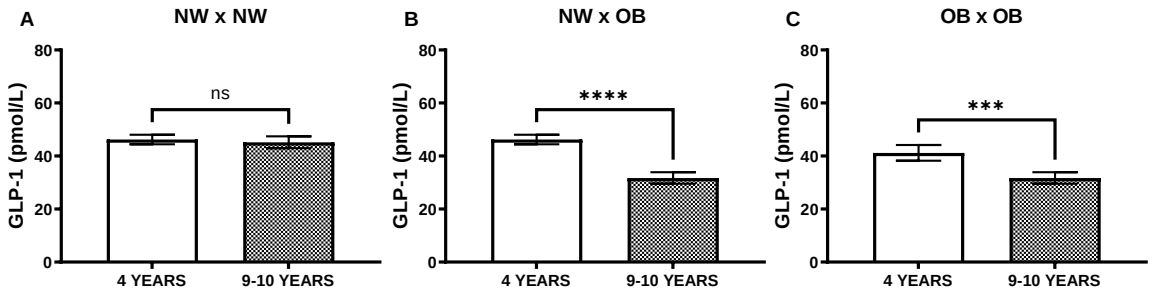

*Figure 5. GLP-1 levels comparison at 4 and 9-10 years old in the tree groups, NWxNW (A); NWxOB (B); OBxOB (C). Values are in mean ± SEM. Wilcoxon test, ****p<0.0001; ***p<0.0005; ns = non-significant (p>0.05). NW = healthy weight children; OB = overweight/ obese children.*

3.3.2 PYY

A mixed effect ANCOVA has shown no effect of time or time x group ($p>0.05$) for PYY levels when comparing the 3 groups at ages 4 vs 9-10 years old (Figure 4A). When the groups were analysed together, independent of BMI, PYY levels showed a reduction with age when children at 4 were compared with children at 9-10 years old (22.30 ± 0.90 vs 16.95 ± 1.06 pmol/L, respectively; $p<0.001$). The delta change analyses for PYY revealed that all 3 groups displayed a reduction on the levels of PYY, however there were no differences in this reduction between groups ($p>0.05$) - Figure 4B. When each group was analysed separately, the NWxOB group (Figure 5B) showed that the levels of PYY were reduced at 9-10 compared to 4 years old. The NWxNW and OBxOB groups displayed a trend for a similar result ($p=0.1$ and $p=0.09$, respectively) - Figure 5A and C.

The data available in the literature shows that PYY levels have a general trend of reducing with maturation. Lloyd et al. (2010), reported that PYY levels reduced in healthy weight females (pre-pubertal vs mid-pubertal) and males (mid-late pubertal vs late pubertal) with maturation. This trend is supported by the work of Patel et al [45] who reported fasting PYY levels to be higher in pre-early pubertal obese children compared to mid-late pubertal obese children. The maximum growth velocity generally seems to occur in girls and boys in the corresponding tanner stage at which PYY levels were the lowest. The data suggest that a decreased fasting PYY could be a potential mechanism facilitating growth by reducing satiety and increasing food intake (Grumbach and Styne 2003; Lloyd et al. 2010; Patel et al., 2014).

The PYY levels from the current cohorts displayed a large inter and intra-individual variability. It is possible that a larger sample size could have provided the power to detect the differences in PYY levels with age. Taken together, the evidence suggest that PYY levels seem to reduce with age, independent of the BMI and weight gain. However, more studies are necessary with an appropriate sample size to confirm these results.

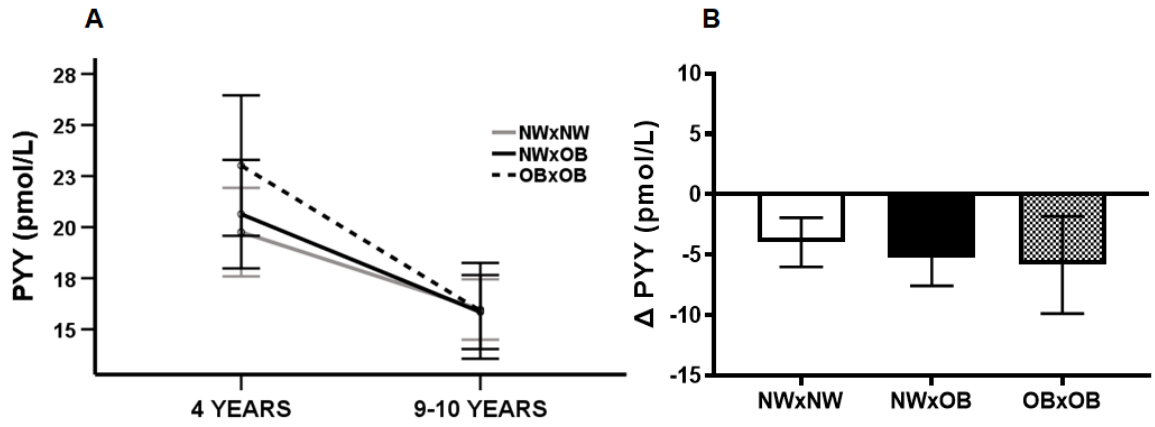

Figure 6. ANCOVA comparison of the PYY levels at 4 and 9-10 years old in the tree groups - NWxNW, NWxOB and OBxOB (A); PYY delta change comparison between the tree groups (B). Values are in mean ± SEM. ANCOVA covariates appearing in the model are evaluated at the following values: G21=1 and Inma=2 = 1.40, effect of time and effect of time x group p>0.05. Kruskal-Wallis test, p>0.05. NW = healthy weight children; OB = overweight/obese children.

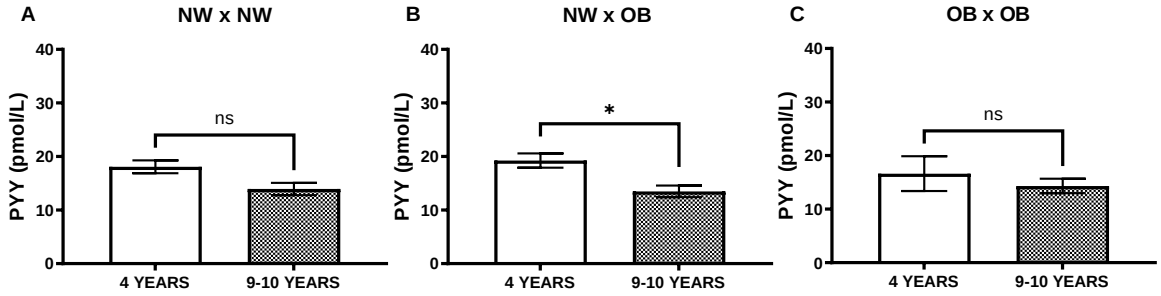

*Figure 7. PYY levels comparison at 4 and 9-10 years old in the tree groups, NWxNW (A); NWxOB (B); OBxOB (C). Values are in mean ± SEM. Wilcoxon test, ****p<0.0001; ***p<0.0005; ns = non-significant (p>0.05). NW = healthy weight children; OB = overweight/ obese children.*

3.3.3 Total Ghrelin

When the total ghrelin levels in each group (NWxNW, NWxOB and OBxOB) were analysed, the tree groups have shown that the levels of GLP-1 were reduced at 10 compared to 4 years old (Figure 8). Studies have compared the total ghrelin levels (Soriano-Guillén et al, 2004; Pomerants et al., 2006; Ellis et al., 2012) and also the acylated (Bellone et al., 2012; Prodam et al., 2011; Patel et al., 2014) and deacylated (Bellone et al., 2012; Prodam et al., 2011) ghrelin isoforms across different pubertal stages. Most of the studies (Soriano-Guillén et al, 2004; Bellone et al., 2012; Pomerants et al., 2006; Prodam et al., 2011; Patel et al., 2014) showed children in the earlier maturation stages had higher levels of ghrelin compared to children in the later maturation stages, and this was true for both healthy weight and obese children. Ellis et al [53], on the other hand, found there to be no difference in fasting total ghrelin levels in peripubertal and pubertal obese females. As for PYY, the reduction on ghrelin levels with age could be a physiological mechanism to favours energy intake and growth.

The results from this study are, therefore, in accordance with the data reported in most of the literature available and show that fasting total ghrelin decrease with age, independent of the BMI and weight gain of the children.

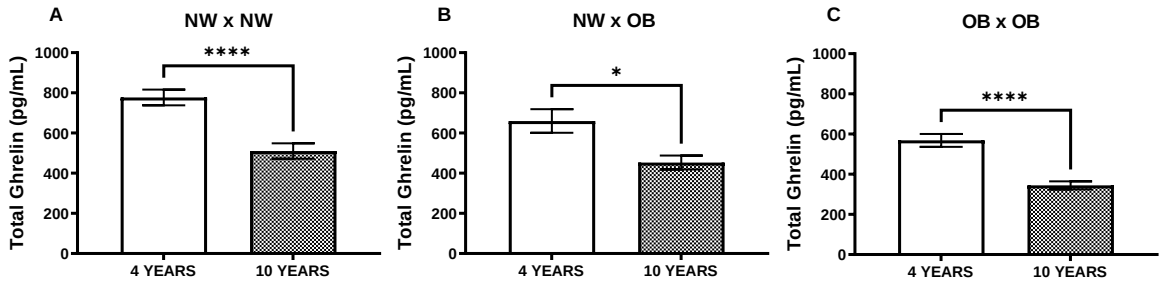

*Figure 8. Total Ghrelin levels comparison at 4 and 9-10 years old in the tree groups, NWxNW (A); NWxOB (B); OBxOB (C). Values are in mean ± SEM. Wilcoxon test, ****p<0.0001; *p<0.05. NW = healthy weight children; OB = overweight/ obese children.*

Parameters	4 YEARS OLD[1]				9-10 YEARS OLD[2]				
	NW	OW/OB	*p*[3]	Total	NW	OW/OB	*p*[4]	Total	*p*[5]
n	316	109		425	233	315		549	
Age (years)	4.46 ± 0.01	4.49 ± 0.03	ns	4.47 ± 0.01	10.47 ± 0.05	10.46 ± 0.04	ns	10.47 ± 0.03	**
Male/Female, n	150/166	54/55		204/221	116/117	178/137			
Body Weight (kg)	17.65 ± 0.11	22.06 ± 0.28	**	18.78 ± 0.14	33.36 ± 0.35	47.56 ± 0.52	**	41.52 ± 0.45	**
Height (cm)	106.2 ± 0.26	108.5 ± 0.44	**	106.8 ± 0.23	140.7 ± 0.46	144.4 ± 0.40	**	142.8 ± 0.31	**
Waist (cm)	51.8 ± 0.17	58.49 ± 0.49	**	53.5 ± 0.22	62.57 ± 0.34	78.23 ± 0.53	**	71.52 ± 0.47	**
BMI z-score	0.22 ± 0.04	2.05 ± 0.09	**	0.69 ± 0.05	-0.122 ± 0.05	1.95 ± 0.03	**	1.07 ± 0.05	**
BMI (kg/m^2)	15.62 ± 0.05	18.68 ± 0.18	**	16.41 ± 0.09	16.75 ± 0.10	22.64 ± 0.17	**	20.13 ± 0.16	**
GLP-1 (pmol/L)	42.95 ± 1.18	39.58 ± 1.84	ns	42.09 ± 0.99	47.09 ± 1.35	39.59 ± 1.40	**	42.74 ± 1.00	ns
PYY (pmol/L)	21.83 ± 0.95	23.62 ± 2.17	ns	22.30 ± 0.90	17.83 ± 2.13	16.28 ± 0.82	ns	16.95 ± 1.06	**
Total Ghrelin (ng/mL)[a]	719.0 ± 35.54	568.4 ± 32.01	*	668.5 ± 26.53	510.6 ± 38.15	398.4 ± 20.59	*	435.6 ± 19.11	**

Table 1. Characteristics of the Study Participants

[1]Cohorts G21, Inma and Environage
[2]Cohorts G21, Inma and Rhea
[3]Comparison between healthy weight and overweight/obese groups at 4 years old. Test Mann-Whitney, **p<0.001, *p<0.01
[4]Comparison between healthy weight and overweight/obese groups at 9-10 years old. Test Mann-Whitney, **p<0.001, *p<0.01
[5]Comparison between total, NW+OW/OB groups, at 4 and 9-10 years old. Wilcoxon test, **p<0.001
[a]Data available for cohort G21 (*n*=150)
NW = healthy weight; OW/OB = overweight/obese; waist = waist circumference; ns = non-significant

4. Conclusions and Recommendations

Effects of Overweight and Obesity on Gut Hormones

In this study, lower fasting total ghrelin levels were shown in overweight and obese compared with normal weight children, independent of age - at 4 and at 10 years old. The same results were also observed for GLP-1 levels in older children at 9-10 years old, but not at the age of 4. PYY levels, on the other hand, do not seem to be affected by BMI, independent of the age. Taken together, the evidence suggests that total ghrelin and GLP-1 levels are dysregulated in overweight and obese youth.

Effects of Gut Hormones on Weight Gain:

The current results suggest that weight gain may have an impact on GLP-1, reducing the hormone levels with age if the children were or became overweight/obese. The data have also shown that fasting total ghrelin and PYY levels seem to decrease with age, but, in this case, the reduction is independent of the BMI and/or weight gain of the children. This reduction on ghrelin and PYY levels may represent a physiological mechanism to favour growth. GLP-1, on the other hand, may play a different role or follow a different control pattern as healthy weight children have clearly not changed their hormone levels with age. The reduction of GLP-1 in the overweight/obese with age may, therefore, signify a disruption on the hormone levels regulation.

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