Title: Mechanisms of Obesity in Prader-Willi Syndrome

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Summary

Obesity is the most common cause of metabolic complications and poor quality of life in Prader-Willi syndrome (PWS). Hyperphagia and obesity develop after an initial phase of poor feeding and failure to thrive. Several mechanisms for the aetiology of obesity in PWS are proposed which include disruption in hypothalamic pathways of satiety control resulting in hyperphagia, aberration in hormones regulating food intake, reduced energy expenditure due to hypotonia and altered behaviour with features of autism spectrum disorder. Profound muscular hypotonia prevents PWS patients from becoming physically active, causing reduced muscle movements and hence reduced energy expenditure. In a quest for the aetiology of obesity, recent evidence has focused on several appetite-regulating hormones, growth hormone, thyroid hormones and plasma adipocytokines. However, despite advancement in understanding of the genetic basis of PWS, there are contradictory data on the role of satiety hormones in hyperphagia and data regarding dietary intake are limited. Mechanistic studies on the aetiology of obesity and its relationship with disease pathogenesis in PWS are required. . In this review, we focused on the available evidence regarding mechanisms of obesity and potential new areas that could be explored to help unravel obesity pathogenesis in PWS.

Introduction

Prader-Willi Syndrome (PWS) is a genetic neurological disorder due to loss of function in the long arm (q11-q13) of paternally derived chromosome 15 occurring in 1 in 16,000 (1 in 10,000 to 1 in 25,000) live births. The loss of function can be caused by a deletion in chromosome 15 (~70-75%), uniparental disomy (UPD) (~20-25%), an imprinting defect due to a mutation in the imprinting centre of the chromosome 15 (~2-5%) or unbalanced translocations (~1%) (1, 2).

The syndrome is characterised prenatally by decreased fetal movements, polyhydromnios and post-natally by hypotonia ("floppy child"), feeding problems, and failure to thrive in early infancy, followed by growth delay, learning difficulties, hyperphagia and obesity, sleep abnormalities, behavioural problems and hypogonadism (1). Characteristic phenotypic features in most but not all PWS patients include short stature, small hands and feet, narrow nasal bridge, almond shaped palpebral fissures, thin upper lip, narrow bifrontal diameter, scoliosis, eye abnormalities, thick saliva, and hypopigmentation (1).

Severe obesity develops in various nutritional stages (3). A classical description of these stages was based on two phases; poor feeding, hypotonia, and failure to thrive in early infancy (phase 1, 0-9 months age), followed by hyperphagia leading to obesity (phase 2, >9 months age to adulthood). However, in a large cohort study of PWS patients followed for 10 years, Miller *et al.* observed a more gradual shift occurring over 7 nutritional phases starting from before birth (phase 0) and continuing into childhood (phase 1a, 1b, 2a, 2b, 3) and adult life (phase 4) (3). These were based on the child's food intake, behaviour, and growth in body mass (Figure 1).

Although Prader Willi syndrome is the most common cause of syndromal obesity, a major cause of metabolic complications and mortality in this group (4), the exact mechanism

for the development of obesity is still largely unknown. Abnormalities in the hypothalamic satiety centre and its hormonal circuitry have been suggested to affect energy expenditure (5), food intake (2), and hormonal deficiencies (2). Other factors implicated include muscle tone (6) and body composition (7). Scoliosis in PWS patients with increasing age is proposed to be the result of prolonged hypotonicity, increasing age, obesity and subtle bone dysplasia rather than growth hormone therapy. However, the interaction of these factors is complex and needs further study. Furthermore, controversial data on the role of satiety hormones, insulin, and plasma adipocytokines suggest that other unknown mechanisms may play a role in the aetiology of obesity in PWS. How far the occurrence of obesity in itself is a confounding risk factor for the distribution of fat and lean mass rather than hormonal aberrations remains to be determined. Diet is an important contributor to the onset and progression of obesity however there are very few studies looking at the dietary intake of PWS patients. This review explores recent evidence related to the hormonal, dietary, and body composition factors related to obesity in PWS. Furthermore, it also suggests potential new areas of research that may help unravel obesity pathogenesis in PWS.

Hormonal hypothalamic regulation of satiety

Several hormones related to central and hypothalamic satiety signals have been studied to explain the aetiology of obesity in PWS (Table 1). Functional magnetic resonance imaging data suggest that PWS patients show greater post-meal sub-cortical (hypothalamus, amygdala, hippocampus) stimulation of food activation centres in the limbic and paralimbic region compared to non-PWS obese and healthy lean controls. In contrast, simple obesity is associated with significantly higher activity in the dorsolateral prefrontal and orbitofrontal cortex associated with inhibitory control of food intake compared to PWS patients (8). This response is even higher for high versus low calorie foods as studies also suggest hyper-stimulation of the satiety related hypothalamic neuronal circuitry in PWS patients compared

to non-PWS obese patients in response to high calorie vs. low calorie foods (9). This indicates that functional dysfunction of reward circuitry regions associated with hypothalamic-satiety-regulating hormones is also involved in development and maintenance of obesity in PWS.

Ghrelin

Ghrelin is a gut hormone which stimulates food intake (orexogenic), growth hormone release, gastric emptying, regulates glucose metabolism, stimulates adipose tissue lipogenesis, and inhibits lipid oxidation (10). Elevated levels of plasma ghrelin stimulate agouti related peptide (AGRP) neurons in the arcuate nucleus of the hypothalamus which in turn inhibit the melanocortin receptor 4 (MCR4) in the paraventricular nucleus of hypothalamus. Inhibition of MCR4 results in delayed satiety and loss of appetite. Persistently increased orexigenic ghrelin levels in PWS, particularly in children after 3-5 years age compared with normal children were first reported by DelParigi and colleagues (11) supported by other studies comparing PWS patients with non-PWS obese, healthy lean, leptin deficient, and melatonin receptor 4 deficient patients (12, 13, 14). In their study, ghrelin levels remained high in PWS patients compared to healthy controls even after the same satiating dose of liquid meals which led to a delayed sense of fullness and persistent drive to eat (11) (Figure 2).

However in contrast, others found no significant difference in plasma ghrelin levels between normal weight PWS patients less than 5 years of age, compared with healthy children matched for age, BMI, and gender (15). This may indicate that levels of ghrelin in PWS patients increase in childhood only prior to the onset of obesity which does not occur in healthy children. This assertion is supported by a study which showed significantly higher levels of plasma ghrelin and a negative correlation between plasma total ghrelin levels and BMI SDS in lean PWS children (median age 3.6 years) compared to lean controls (16). In a

recent study of sixty very young (<2 years age) PWS patients in the early nutritional phase (phase 1), plasma ghrelin levels were significantly higher than in healthy early-onset morbidly obese patients and healthy sibling lean controls (17). Higher levels of ghrelin were observed in these patients in early nutritional phases (phase 1a and 1b) long before the onset of hyperphagia which suggests that higher plasma ghrelin may not be causally related to the onset of hyperphagia (17). Ghrelin up-regulates adipose tissue lipogenesis and inhibits lipolysis by activating sterol response element binding proteins, acyl CoA carboxylase, lipoprotein lipase, and fatty acid synthase independent of its orexigenic effects (18). Whether persistent increases in plasma ghrelin are involved in triggering higher fat mass in PWS and whether the effect of growth hormone on fat mass is due to suppression of the plasma ghrelin; needs further research.

Insulin

Plasma insulin deficient states or insulin resistance cause diabetes mellitus, and up to 20% of PWS children develop type 2 diabetes (19). Insulin inhibits neuropeptide Y and stimulates pro-opiomelanocortin (POMC) neurons in the arcuate nucleus to reduce food intake and is regarded as one of the mechanisms contributing to obesity in PWS. Some evidence suggests lower fasting plasma insulin and delayed insulin secretion during an oral glucose tolerance test (OGTT) with or without normal insulin sensitivity (20), while others have suggested increased plasma insulin depicting insulin resistance (21) (Table 1). When compared with age, weight, and BMI matched non-PWS obese controls, obese PWS subjects manifest different glucoregulatory mechanisms via reduced β-cell response to glucose stimulation, a significantly increased hepatic insulin extraction, and dissociation of obesity and insulin resistance (22). Obesity is a diabetogenic state, therefore it is unclear whether changes in insulin levels are a consequence of severe obesity or the insulin secreting capability of PWS

patients is abnormal (20). Plasma insulin is an inhibitor of ghrelin independent of plasma glucose levels (23). Reduced insulin levels in diabetic PWS patients may therefore be a contributory factor to the elevated plasma ghrelin and its hypothalamic effects.

Growth hormone

Deficiency of growth hormone (GH) in PWS is associated with low muscle mass, increased fat mass, poor muscle tone and strength, decreased movements, and reduced energy expenditure and exercise tolerance (24). GH replacement therapy in adult PWS patients is associated with an increase in skeletal muscle mass, reduction in percentage body fat, increased muscle tone and exercise endurance, independent of the growth hormone secretory status (25). Furthermore, higher systemic inflammatory cytokines such as TNFα, MCP-1, and IL-8 and significantly lower fasting glycaemia, insulinemia, IGF-1, and HOMA-IR values have been shown to partially reverse with GH replacement therapy compared to non-PWS obese controls. Compared to untreated patients Tanner stage 1 and 2, GH replacement therapy seems to improve mean energy intake and reduce total body fat mass measured by DEXA despite higher saturated fat intake (26). This might indicate improved metabolism and energy expenditure with GH treatment. Moreover, studies following patients for 12-24 months after the cessation of GH replacement have shown a progressive increase in BMI and a tendency towards an increase in visceral adipose tissue (27).

Obestatin

Obestatin is produced in the stomach by post-translational modification of ghrelin. In contrast to ghrelin, obestatin suppress food intake, inhibits gastric emptying, and decrease weight gain (28). Unlike ghrelin, obestatin binds to a G protein coupled receptor 39 (GPR39) although it does not cross the blood brain barrier (28). No study has reported significant difference in plasma obestatin levels between obese PWS and obese non-PWS patients (29).

Plasma Adipocytokines

Leptin

Leptin reduces food intake and energy metabolism by inhibiting neuropeptide Y neurons in the arcuate nucleus. Although plasma leptin in PWS patients is positively correlated with BMI and body fat mass, no difference has been found in leptin concentration in PWS infants (17), children and adults (30) compared to healthy normal weight and obese when adjusted for BMI or fat mass. Although, significantly higher leptin mRNA and plasma leptin concentration in obese PWS and non-PWS obese children compared to healthy non-obese children was also reported in a small number of patients (n=6 in each group) (31). No difference in the relationship of leptin mRNA levels between PWS and non-PWS obesity might suggest similar response of leptin to obesity regardless of its cause. Whether the hypothalamic response to the levels of leptin is also the same, needs to be investigated.

Amongst other adipocytokines, plasma resistin and adiponectin have been studied in PWS obese and non-obese patients (32, 33) (Table 1). Higher levels of resistin are associated with insulin resistance and lipogenesis in PWS obese patients (32) while plasma adiponectin is anti-inflammatory, anti-atherogenic and associated with increased insulin sensitivity in PWS patients (33).

Visfatin, produced by adipose tissue, is positively associated with systemic inflammation, atherogenesis, and diabetes (34) and increases by up to 32% for each hour decrease in rapid eye movement (REM) sleep (35). PWS patients with obesity have reduced REM sleep and are therefore at risk of increased plasma adipocytokines. However, visfatin has not yet been measured in PWS.

Peptide YY

Peptide YY is released from ileal and colonic cells postprandially to induce satiety by stimulating POMC neurons, inhibiting NPY, and reducing gastric emptying (Table 1, Figure 2). There are two isoforms; PYY (1-36), selective for NPY1, 2, and 5 receptors, and PYY (3-

36), an anorectic sub-type, highly selective for NPY2 receptor in the arcuate nucleus which regulates food intake under physiological conditions (36). There is contradictory evidence suggesting reduced (14) or increased (37) levels of PYY (3-36) in obese PWS compared to non-PWS obese and lean controls.

Thyroid hormones

Approximately 20-30 % of PWS patients suffer from deficiency in central hypothalamic thyroid hormone-releasing hormone at birth (1, 38) and up to 2 years of age (38). Reduced free, total T4, T3, and TSH suggests disturbance of the hypothalamic thyroid-releasing hormone and TSH axis. Hypothyroidism from early infancy adds to the floppiness, hypotonia, reduced energy expenditure and reduced BMR and hence obesity in later years.

In summary, alteration in several satiety and peripheral satiety hormones may affect the hypothalamic satiety regulation in PWS resulting in delayed satiety and early appetite stimulation (Table 1). Furthermore, the peripheral effects of growth and thyroid hormone deficiency affect body composition contributing to reduced energy expenditure. Contradictory data on the relationship of body fat mass and BMI in PWS and non-PWS obese patients raises the question as to whether satiety hormones are causatively related to the aetiology of hyperphagia in PWS.

Dietary intake in PWS

Obesity results from an imbalance between energy intake and expenditure. Diet is therefore likely to be an important contributory factor. Although reduced energy expenditure and hypothalamic dysfunction might promote energy accumulation in PWS children and young adults, the occurrence of in-satiable hunger and gastroparesis might promote dietary intake (39). “Hypo-activity” and “hypo-metabolism” in PWS children requires intake of 20-30% lower energy than healthy age-matched children. Adherence to specific macronutrient and

energy restricted diets reduces the proportion of body fat (19.8% vs. 41.9%) and body mass index (0.3 SDS vs. 2.23 SDS) in children and adults (40).

Although the effect of dietary intervention on the body composition of PWS patients has been investigated, very few studies have looked at actual daily dietary intake in obese PWS children. Furthermore, none has compared dietary intake between healthy obese and obese-PWS groups of the same age range which could give an indication whether PWS obese patients under-report or under-eat similar to the healthy obese.

An early study by Holm and Pipes (1976) on 14 PWS patients reported an intake of 650-1050 Kcal/day during the initial period of weight loss depending on the size of the patient (41). Eight of 11 patients who lost weight were able to successfully maintain their weight over 6 months to 5 years on a 800-1990 Kcal/day diet appropriate for age (41). This suggests that hyperphagia and subsequent obesity can be prevented by restriction of caloric intake. Moreover, children below 5 years with PWS report a daily energy intake of approximately 30% to 65% below recommended amounts followed for up to 3 years (42). Similar results have been observed in adults with reported daily energy intake of 1000-1500 kcal (43).

These studies are limited by subject numbers, narrow age range, limited time of dietary data collection, not accounting for age related differences in dietary intake, and dietary intake reported by parents. Recording reliable dietary information in PWS patients with behavioural issues is a challenge. Intake of a balanced nutritious diet is essential for normal growth and homeostasis. This suggests consideration of appropriate nutritional support tailored to individuals and not just energy restriction. Further large scale studies with more robust methods of recording dietary data are needed to record the routine nutrient intake of these patients before dietary intervention strategy is applied to ensure balanced growth, preventing obesity and under-nutrition of the patients at the same time.

Body composition in PWS

Obesity attributed to no known identifiable cause has been shown to differ from hypothalamic obesity in PWS in terms of both intrinsic (such as GH, thyroid hormones, insulin, and leptin) and extrinsic factors (such as exercise, diet, and lifestyle). Growth hormone deficiency, hypothyroidism and hypogonadism in addition to lower energy expenditure (both resting and activity), hypotonia, and behavioural issues in patients with PWS result in lower lean mass by 25-27% and a higher fat mass compared to simple obese patients (44). Reduced lean mass with lower physical activity and muscular hypotonia could result in less weight-bearing stress on the bones and hence lower bone-mineral content and density (45) particularly after adjustment for height and age of the patient. This suggests that differences in lean mass, fat mass or bone-mineral density should also be studied in the context of height for age of the patients and their pituitary status. The distribution of fat and lean mass differ between body sites (e.g. between lumber & spine area and the hips & thighs) indicates the need for careful interpretation of body composition measurements. How far the occurrence of obesity in itself is a confounding risk factor for fat and lean mass distribution rather than hormonal aberrations, remains to be determined. Long-term follow up studies are therefore required to characterize the changes in body composition in PWS patients.

Genetic variants in relation to obesity in PWS

Of the three main molecular mechanisms of PWS genotypes (deletion, UPD 15, and imprinting defects), no significant difference in the prevalence of obesity or hyperphagia between the deletion or non-deletion PWS patients have been reported (46). Although no peculiar characteristic can exclusively be attributed to individual genotype, psychiatric illness and intellectual disability is more common in mUPD compared to need for special feeding techniques, sleep disturbance, hypopigmentation, and speech articulation defects in the

deletion group (47). Although individual cases have been reported suggesting association of hyperphagia, obesity and hypogonadism with specific genetic aberrations such as microdeletions of HBII-85 class of small nucleolar RNAs (snoRNAs) (48), lack of expression of PWCR1/HBII-85 snoRNAs (49), and SNORD116 C/D box snoRNA cluster (50), there is scarcity of mechanistic evidence from mutant animal models that could prove the effect of these aberrations on obese/lean phenotype.

Patients with UPD have been observed with significantly lower insulin-induced growth hormone secretion compared to the deletion group (51). However there was no significant difference in the yearly improvement in height (52) or the bone-mineral density (53) in response to GH replacement therapy in either group. The lack of significant obese phenotype-genotype correlation and a similar response to GH despite differences in basal GH secretion suggests that PWS children acquire obesity regardless of the genetic cause and that obesity results from a constellation of behavioral, psychiatric, and developmental disturbances.

Physical activity and behaviour in PWS

With characteristic disease-related muscle hypotonia and alteration in body composition, differences in physical activity between obese PWS and obese non-PWS patients or the healthy population are expected. Evidence suggests reduced physical activity (by ~20%) and reduced vigour (by ~30%) in PWS obese versus non-PWS obese subjects (6). Only 12% patients reach local recommendations for daily physical activity compared with 20-22% of the normal population (54). Interestingly, this physical activity level is independent of adiposity.

Long term home based exercise interventions improve lean muscle mass, reduce calf skinfold and increase spontaneous physical activity (from 45% to 71%), and exercise capacity (from 31% to 78%) (55).

Autistic features are present in up to 36% PWS patients and could be due to the overexpression of ubiquitin protein ligase E3A (UBE3A) in maternal UPD, which significantly contributes to mental retardation and behavioural and communication problems (56). These traits tend to increase with age (56) and may contribute to overweight and obesity by increasing dietary intake and reduce physical activity due to a "lonely" and less socializing behaviour.

Patients with PWS frequently suffer from daytime sleepiness and have abnormal circadian rhythms of rapid eye movement sleep, central hypoventilation, abnormal ventillatory response to hypoxia, and hypercapnia. This leads to episodes of apnoea and hypopnea and disturbed sleep further exacerbated by obesity. Constellation of these disorders lead to reduced physical activity and energy expenditure, anxiety, stereotyped behaviour, difficulty in maintaining social relations and communication (57).

Conclusions and future directions

Obesity is the leading cause of morbidity and mortality in PWS patients. It is a complex phenomenon occurring due to disturbance in the hypothalamic satiety regulatory mechanisms contributed by several hormones, body composition differences, low physical activity, altered feeding behaviour and increased dietary intake (supplementary figure 1). However, the exact mechanisms responsible remain to be determined and need further study.

Obesity in PWS is associated with chronic low-grade inflammation which is not explained by obesity and insulin resistance (58). The gut microbiota have been recently suggested to be involved in obesity-genesis via increased energy harvest from fermentable carbohydrates. The gut microbiota in non-PWS obesity have also been associated with chronic low-grade inflammation. However, this has not been studied in obese PWS patients. There is limited evidence of baseline dietary habits of PWS patients and therefore

longitudinal studies are needed to elucidate the dietary patterns of these patients to individually tailor dietary intervention.

Conflict of Interest: None

Authors' contribution: MJK wrote the review. MGS, CAE, and KG supervised MJK and reviewed the paper.

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Figure Legends

Figure 1: Obesity in relation to nutritional phases in Prader-Willi syndrome.

PWS children are hypotonic with poor suck and failure to thrive in early infancy but gradually catch up with their growth in phase 2a and 2b. Obesity develops by phase 3 when most of the factors contributing to obesity have already set in. Some patients develop obesity very early (e.g. during phase 2a) (Miller *et al* 2011) (course shown in dotted line). NIDDM; Non-insulin dependent diabetes mellitus, m; months, y; years

Figure 2: Mechanism of obesity in Prader Willi Syndrome. Adapted from Mutch and Karine (2006) (59).

Decreased plasma insulin and PYY result in loss of stimulatory signals to the POMC neurons and loss of inhibitory signals to NPY neurons in the arcuate nucleus which fails to stimulate α and β-MSH to control satiety via activation of MCR4 receptor in the Paraventricular nucleus. The role of leptin is still under investigation (marked with "?" in the figure) as overall evidence suggests no difference in leptin concentration in PWS obese vs. non-PWS obese. On the other hand, persistent increase in plasma ghrelin results in stimulation of neurons expressing NPY and AGRP which inhibit MCR4 signalling and hence increase drive towards food intake (3). Alteration in TRH-TSH axis results in reduced energy expenditure (2). Deficiency of GH due to loss of feedback mechanism despite persistent increase in plasma ghrelin results in growth delay increasing weight for height ratio, reduced muscle mass, and increased body fat (1). AGRP, agouti-related protein; α-MSH, alpha melanocyte stimulating hormone receptor; NPY, neuropeptide Y; POMC, pro-opiomelanocortin;TRKB, tyrosine kinase receptor, GH; growth hormone, PYY; Peptide YY, TRH; Thyroid hormone releasing hormone, TSH; Thyroid stimulating hormone, TRKB; Tyrosine kinase receptor B.

Supplementary figure 1: **Simplified scheme for the mechanism of obesity in Prader Willi syndrome**

GH; Growth hormone, TSH-TRH; thyroid stimulating hormone-thyroid releasing hormone, EE; energy expenditure, BMR; basal metabolic rate

354 Table 1: Hormones related to aetiology of obesity in Prader-Willi Syndrome

Hormone	Site of Production	Site of Action	Physiological Role	Pathology in PWS	Ref
Ghrelin	Stomach	AGRP in Arcuate nucleus, adipose tissues	Regulates short term food intake, ↑ in hunger, ↓ after food intake,	Persistently ↑ ghrelin even after food intake leading to weight gain. Levels vary with age	(11)
			Regulates lipid metabolism	↑ body fat	
			↑ GH secretion	Failure to increase GH leading to growth delay, failure to thrive, short stature	
Obestatin	Derived post-transnationally from preproghrelin	AGRP in Arcuate nucleus	Suppresses appetite, inhibit jejunal contractions, and decrease body weight	Limited evidence, Higher Obestatin in ≤3 years PWS patients contributing to failure to thrive and poor feeding in early stages	(60)
				No difference between obese PWS and obese controls	(29)
Leptin	Adipose tissue	POMC and NPY neurons in arcuate nucleus	Primarily inhibits NPY but also stimulates POMC neurons leading to stimulation of MCR4 receptor to induce satiety	Levels similar in PWS and obese control although positively correlated with BMI and body fat	(30)
Resistin	Adipose tissue	Liver	Hepatic insulin resistance and lipogenesis	↑ in PWS (not related to insulin resistance, only related to the degree of	(32)

				obesity)	
				No difference between obese PWS and obese and lean controls	(33)
Adiponectin	Adipose tissue	β-cells in pancreas	↑ Insulin sensitivity , anti-inflammatory, anti-atherogenic	↑ in PWS compared to non-PWS obese, significant positive correlation with insulin sensitivity in PWS but not in obese controls	(33)
				↑ in PWS compared to obese controls but ↓ in PWS compared to lean, no correlation with insulin sensitivity and anthropometric measurements	(37)
Visfatin	Adipose tissue	Pancreas, muscles, liver	Associated with inflammation and insulin resistance. Increase with short sleep duration	No data available	
PYY	Duodenum	Inhibitory Presynaptic receptor for NPY	Induce satiety by stimulating POMC and inhibiting NPY resulting in dis-inhibition of α and β MSH	↓ PYY (3-36) in PWS compared to healthy controls leading to delayed sense of fullness	(14)
			Reduce gastric emptying and gut transit time	Delayed sense of fullness, overeating	
				↑ in PWS compared to non-PWS obese,	(61)
				↑ in PWS compared to obese controls but ↓ in PWS compared to lean. No	(37)

				correlation with insulin sensitivity and anthropometric measurements	
Insulin	Pancreas	POMC and NPY neurons in arcuate nucleus	Stimulate POMC and inhibit NPY neurons leading to stimulation of MCR4 receptor to induce satiety	↓ in PWS leading to hyperphagia, and NIDDM in adulthood	(61)
Growth Hormone	Anterior pituitary	Muscles, Bones, adipose tissue	Induces normal growth and energy metabolism	Growth delay, altered metabolism and energy expenditure	(24, 25)
GLP-1	Intestine	Pancreas	Enhances insulin sensitivity	No difference at baseline, ↑ after GH replacement therapy	(33)
Thyroid hormones	Thyroid gland	Muscles, Bones, adipose tissue	Regulate whole body metabolism	↓ in PWS resulting in altered metabolic rate and energy expenditure	(38)

355 GLP-1; Glucagon-like peptide 1, AGRP; Agouti-related peptide, GH; growth hormone, POMC; pro-opiomelanocortin, NPY; neuropeptide Y,
356 NIDDM; non-insulin dependent diabetes mellitus, PWS; Prader-Willi syndrome.

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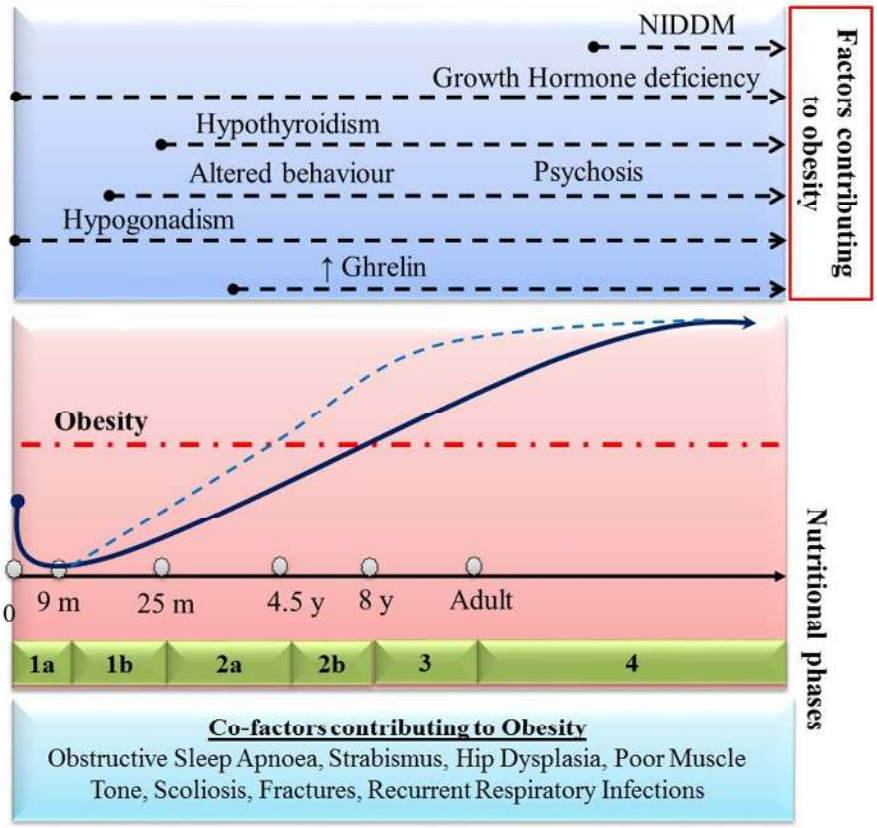

NIDDM
Growth Hormone deficiency
Hypothyroidism
Altered behaviour
Psychosis
Hypogonadism
↑ Ghrelin
Factors contributing to obesity
Obesity
0
9 m
25 m
4.5 y
8 y
Adult
1a
1b
2a
2b
3
4
Nutritional phases
Co-factors contributing to Obesity
Obstructive Sleep Apnoea, Strabismus, Hip Dysplasia, Poor Muscle Tone, Scoliosis, Fractures, Recurrent Respiratory Infections

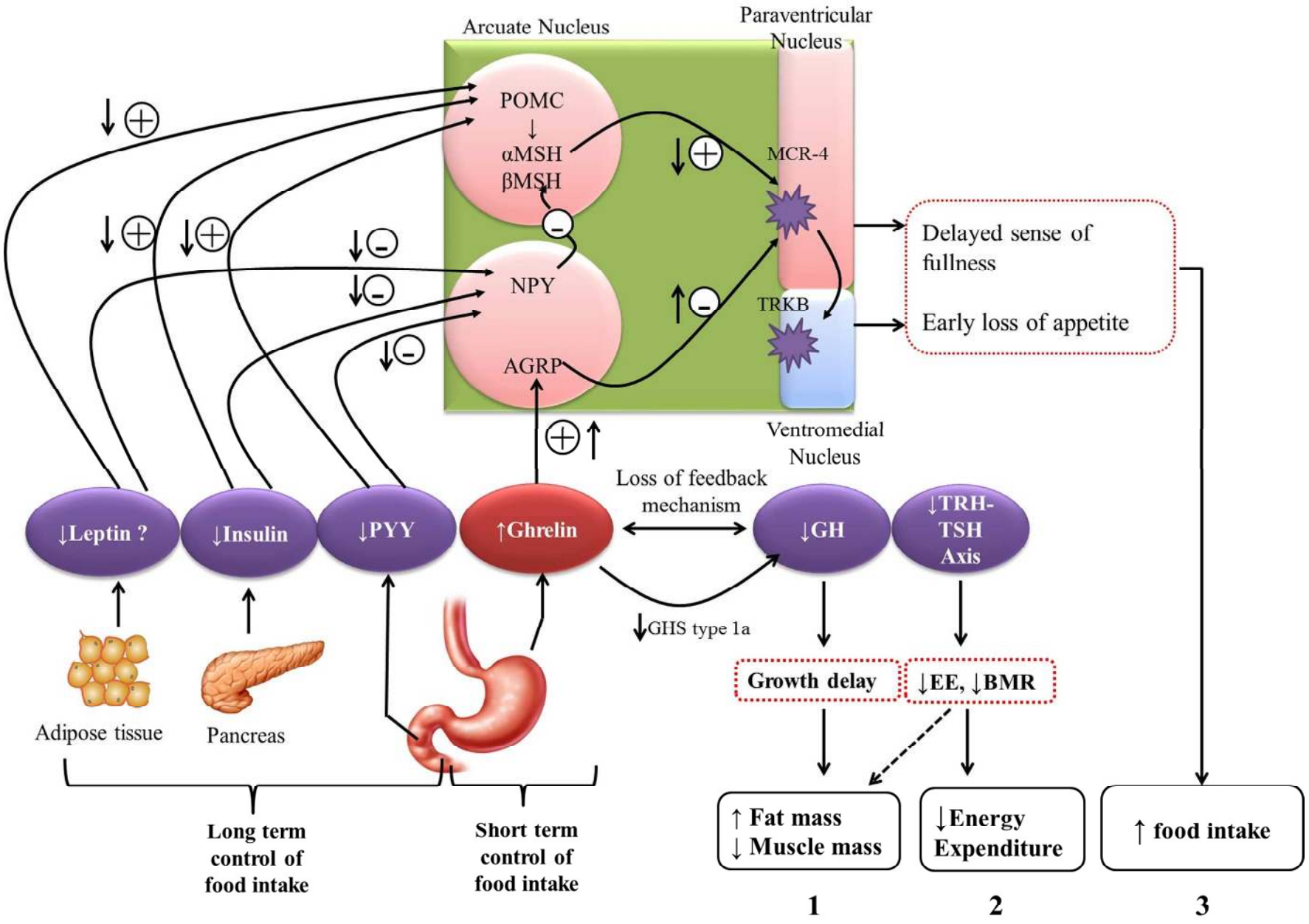

Arcuate Nucleus
Paraventricular Nucleus
POMC
αMSH
βMSH
NPY
AGRP
MCR-4
TRKB
Delayed sense of fullness
Early loss of appetite
Ventromedial Nucleus
Loss of feedback mechanism
↓Leptin ?
↓Insulin
↓PYY
↑Ghrelin
↓GH
↓TRH-TSH Axis
↓GHS type 1a
Adipose tissue
Pancreas
Growth delay
↓EE, ↓BMR
↑ Fat mass
↓ Muscle mass
↓Energy Expenditure
↑ food intake
Long term control of food intake
Short term control of food intake
1
2
3

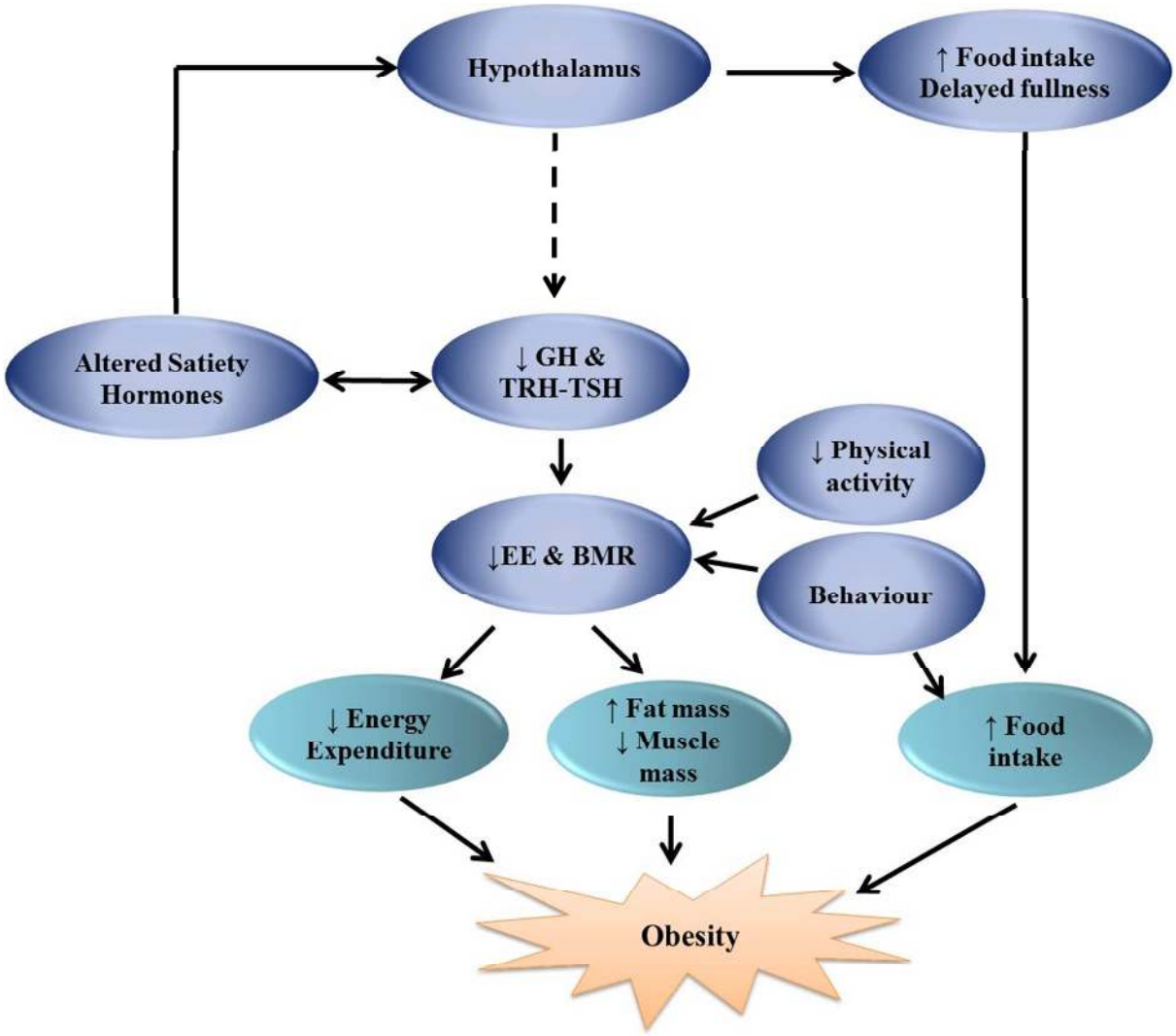
Table 1: Hormones related to aetiology of obesity in Prader-Willi Syndrome

Hormone	Site of Production	Site of Action	Physiological Role	Pathology in PWS	Ref
Ghrelin	Stomach	AGRP in Arcuate nucleus, adipose tissues	Regulates short term food intake, ↑ in hunger, ↓ after food intake,	Persistently ↑ ghrelin even after food intake leading to weight gain. Levels vary with age	(11)
			Regulates lipid metabolism	↑ body fat	
			↑ GH secretion	Failure to increase GH leading to growth delay, failure to thrive, short stature	
Obestatin	Derived post-transnationally from preproghrelin	AGRP in Arcuate nucleus	Suppresses appetite, inhibit jejunal contractions, and decrease body weight	Limited evidence, Higher Obestatin in ≤3 years PWS patients contributing to failure to thrive and poor feeding in early stages	(60)
				No difference between obese PWS and obese controls	(29)
Leptin	Adipose tissue	POMC and NPY neurons in arcuate nucleus	Primarily inhibits NPY but also stimulates POMC neurons leading to stimulation of MCR4 receptor to induce satiety	Levels similar in PWS and obese control although positively correlated with BMI and body fat	(30)
Resistin	Adipose tissue	Liver	Hepatic insulin resistance and lipogenesis	↑ in PWS (not related to insulin resistance, only related to the degree of obesity)	(32)

				No difference between obese PWS and obese and lean controls	(33)
Adiponectin	Adipose tissue	β-cells in pancreas	↑ Insulin sensitivity , anti-inflammatory, anti-atherogenic	↑ in PWS compared to non-PWS obese, significant positive correlation with insulin sensitivity in PWS but not in obese controls	(33)
				↑ in PWS compared to obese controls but ↓ in PWS compared to lean, no correlation with insulin sensitivity and anthropometric measurements	(37)
Visfatin	Adipose tissue	Pancreas, muscles, liver	Associated with inflammation and insulin resistance. Increase with short sleep duration	No data available	
PYY	Duodenum	Inhibitory Presynaptic receptor for NPY	Induce satiety by stimulating POMC and inhibiting NPY resulting in dis-inhibition of α and β MSH	↓ PYY (3-36) in PWS compared to healthy controls leading to delayed sense of fullness	(14)
			Reduce gastric emptying and gut transit time	Delayed sense of fullness, overeating	
				↑ in PWS compared to non-PWS obese,	(61)
				↑ in PWS compared to obese controls but ↓ in PWS compared to lean. No correlation with insulin sensitivity and anthropometric measurements	(37)

Insulin	Pancreas	POMC and NPY neurons in arcuate nucleus	Stimulate POMC and inhibit NPY neurons leading to stimulation of MCR4 receptor to induce satiety	↓ in PWS leading to hyperphagia, and NIDDM in adulthood	(61)
Growth Hormone	Anterior pituitary	Muscles, Bones, adipose tissue	Induces normal growth and energy metabolism	Growth delay, altered metabolism and energy expenditure	(24, 25)
GLP-1	Intestine	Pancreas	Enhances insulin sensitivity	No difference at baseline, ↑ after GH replacement therapy	(33)
Thyroid hormones	Thyroid gland	Muscles, Bones, adipose tissue	Regulate whole body metabolism	↓ in PWS resulting in altered metabolic rate and energy expenditure	(38)

GLP-1; Glucagon-like peptide 1, AGRP; Agouti-related peptide, GH; growth hormone, POMC; pro-opiomelanocortin, NPY; neuropeptide Y, NIDDM; non-insulin dependent diabetes mellitus, PWS; Prader-Willi syndrome.


Hypothalamus
↑ Food intake
Delayed fullness
Altered Satiety
Hormones
↓ GH &
TRH-TSH
↓ Physical
activity
↓EE & BMR
Behaviour
↓ Energy
Expenditure
↑ Fat mass
↓ Muscle
mass
↑ Food
intake
Obesity

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4. Expert testimony	X					× ADD
5. Grants/grants pending	X					× ADD
6. Payment for lectures including service on speakers bureaus	X					× ADD
7. Payment for manuscript preparation	X					×

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Relevant financial activities outside the submitted work

Type of Relationship (in alphabetical order)	No	Money Paid to You	Money to Your Institution*	Entity	Comments	
						ADD
8. Patents (planned, pending or issued)	X					×
						ADD
9. Royalties	X					×
						ADD
10. Payment for development of educational presentations	X					×
						ADD
11. Stock/stock options	X					×
						ADD
12. Travel/accommodations/ meeting expenses unrelated to activities listed**	X					×
						ADD
13. Other (err on the side of full disclosure)	X					×
						ADD

* This means money that your institution received for your efforts.
** For example, if you report a consultancy above there is no need to report travel related to that consultancy on this line.

Section 4. Other relationships

Are there other relationships or activities that readers could perceive to have influenced, or that give the appearance of potentially influencing, what you wrote in the submitted work?

- [x] No other relationships/conditions/circumstances that present a potential conflict of interest
- [ ] Yes, the following relationships/conditions/circumstances are present (explain below):

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Instructions

The purpose of this form is to provide readers of your manuscript with information about your other interests that could influence how they receive and understand your work. The form is designed to be completed electronically and stored electronically. It contains programming that allows appropriate data display. Each author should submit a separate form and is responsible for the accuracy and completeness of the submitted information. The form is in four parts.

1. Identifying information.

Enter your full name. If you are NOT the corresponding author please check the box "no" and a space to enter the name of the corresponding author in the space that appears. Provide the requested manuscript information. Double-check the manuscript number and enter it.

2. The work under consideration for publication.

This section asks for information about the work that you have submitted for publication. The time frame for this reporting is that of the work itself, from the initial conception and planning to the present. The requested information is about resources that you received, either directly or indirectly (via your institution), to enable you to complete the work. Checking "No" means that you did the work without receiving any financial support from any third party -- that is, the work was supported by funds from the same institution that pays your salary and that institution did not receive third-party funds with which to pay you. If you or your institution received funds from a third party to support the work, such as a government granting agency, charitable foundation or commercial sponsor, check "Yes". Then complete the appropriate boxes to indicate the type of support and whether the payment went to you, or to your institution, or both.

3. Relevant financial activities outside the submitted work.

This section asks about your financial relationships with entities in the bio-medical arena that could be perceived to influence,or that give the appearance of potentially influencing, what you wrote in the submitted work. You should disclose interactions with ANY entity that could be considered broadly relevant to the work. For example, if your article is about testing an epidermal growth factor receptor (EGFR) antagonist in lung cancer, you should report all associations with entities pursuing diagnostic or therapeutic strategies in cancer in general, not just in the area of EGFR or lung cancer.

Report all sources of revenue paid (or promised to be paid) directly to you or your institution on your behalf over the 36 months prior to submission of the work. This should include all monies from sources with relevance to the submitted work, not just monies from the entity that sponsored the research. Please note that your interactions with the work's sponsor that are outside the submitted work should also be listed here. If there is any question, it is usually better to disclose a relationship than not to do so.

For grants you have received for work outside the submitted work, you should disclose support ONLY from entities that could be perceived to be affected financially by the published work, such as drug companies, or foundations supported by entities that could be perceived to have a financial stake in the outcome. Public funding sources, such as government agencies, charitable foundations or academic institutions, need not be disclosed. For example, if a government agency sponsored a study in which you have been involved and drugs were provided by a pharmaceutical company, you need only list the pharmaceutical company.

4. Other relationships.

Use this section to report other relationships or activities that readers could perceive to have influenced, or that give the appearance of potentially influencing, what you wrote in the submitted work.

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Section 1. Identifying Information

1. Given Name (First Name): M. GUFTAR
2. Surname (Last Name): SHAIKH
3. Effective Date (07-August-2008): 22 JULY 2016

4. Are you the corresponding author? ☑ Yes ☐ No

5. Manuscript Title: MECHANISMS OF OBESITY IN PRADER-WILLI SYNDROME

6. Manuscript Identifying Number (if you know it)

Section 2. The Work Under Consideration for Publication

Did you or your institution at any time receive payment or services from a third party for any aspect of the submitted work (including but not limited to grants, data monitoring board, study design, manuscript preparation, statistical analysis, etc...)?

Complete each row by checking "No" or providing the requested information. If you have more than one relationship click the "Add" button to add a row. Excess rows can be removed by clicking the "X" button.

The Work Under Consideration for Publication

Type	No	Money Paid to You	Money to Your Institution*	Name of Entity	Comments**	
1. Grant	☑	☐	☐			X ADD
2. Consulting fee or honorarium	☑	☐	☐			X ADD
3. Support for travel to meetings for the study or other purposes	☑	☐	☐			X ADD
4. Fees for participation in review activities such as data monitoring boards, statistical analysis, end point committees, and the like	☑	☐	☐			X ADD
5. Payment for writing or reviewing the manuscript	☑	☐	☐			X ADD
6. Provision of writing assistance, medicines, equipment, or administrative support	☑	☐	☐			X

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The Work Under Consideration for Publication

Type	No	Money Paid to You	Money to Your Institution*	Name of Entity	Comments**	
7. Other	☑	☐	☐			ADD / X / ADD

* This means money that your institution received for your efforts on this study.
** Use this section to provide any needed explanation.

Section 3. Relevant financial activities outside the submitted work.

Place a check in the appropriate boxes in the table to indicate whether you have financial relationships (regardless of amount of compensation) with entities as described in the instructions. Use one line for each entity; add as many lines as you need by clicking the "Add +" box. You should report relationships that were present during the 36 months prior to submission.

Complete each row by checking "No" or providing the requested information. If you have more than one relationship click the "Add" button to add a row. Excess rows can be removed by clicking the "X" button.

Relevant financial activities outside the submitted work

Type of Relationship (in alphabetical order)	No	Money Paid to You	Money to Your Institution*	Entity	Comments	
1. Board membership	☑	☐	☐			X / ADD
2. Consultancy	☑	☐	☐			X / ADD
3. Employment	☑	☐	☐			X / ADD
4. Expert testimony	☑	☐	☐			X / ADD
5. Grants/grants pending	☑	☐	☐			X / ADD
6. Payment for lectures including service on speakers bureaus	☑	☐	☐			X / ADD
7. Payment for manuscript preparation	☑	☐	☐			X

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Relevant financial activities outside the submitted work

Type of Relationship (in alphabetical order)	No	Money Paid to You	Money to Your Institution*	Entity	Comments	
8. Patents (planned, pending or issued)	☑	☐	☐			ADD / X
9. Royalties	☑	☐	☐			ADD / X
10. Payment for development of educational presentations	☑	☐	☐			ADD / X
11. Stock/stock options	☑	☐	☐			ADD / X
12. Travel/accommodations/ meeting expenses unrelated to activities listed**	☑	☐	☐			ADD / X
13. Other (err on the side of full disclosure)	☑	☐	☐			ADD / X

* This means money that your institution received for your efforts.
** For example, if you report a consultancy above there is no need to report travel related to that consultancy on this line.

Section 4. Other relationships

Are there other relationships or activities that readers could perceive to have influenced, or that give the appearance of potentially influencing, what you wrote in the submitted work?

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