Motor development in Prader-Willi Syndrome

For reasons of consistency within this thesis, some terms have been standardized throughout the text. As a consequence the text may differ in this respect from the articles that have been published.

The studies presented in this thesis have been performed at the Scientific Institute for Quality of Healthcare (IQ healthcare) and the department of Rehabilitation, Pediatric Physical Therapy. These are part of the Nijmegen Centre of Evidence Based Practice (NCEBP), one of the approved research institutes of the Radboud University Nijmegen Medical Centre.

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1

General introduction

Introduction

This thesis focuses on motor development in infants with Prader-Willi syndrome (PWS) and the effect of child-specific physical training combined with growth hormone (GH) treatment on motor development.

Prader, Labhart, and Willi first described PWS in 1956[1] as a clinical syndrome. PWS is a rare genetic multisystem disorder resulting from the lack of expression of the paternally derived chromosome 15. The estimated prevalence is 1 in 10,000–30,000 live births.[2] The most significant characteristics are hypotonia, hyperphagia, obesity, short stature, mild dysmorphic facial features, cognitive and behavioral deficits, and endocrine disturbances like hypogonadism and GH deficiency.[3] In infancy, feeding difficulties, failure to thrive, severe muscular hypotonia, and muscle weakness result in serious developmental delay.[4-6]

Although the motor problems in infancy are distinctive characteristics of PWS and of great concern for parents, clinical studies focusing on early motor development in PWS are lacking. As a result, insight into the motor development pattern in these infants is limited and information concerning interventions to improve their motor development is scarce. Despite many descriptions of symptoms, the causes of hypotonia, muscle weakness, and motor problems in PWS are still not clear. It is presumed that abnormal body composition in PWS patients with increased fat mass and reduced muscle mass, and possibly some degree of neuromuscular abnormality are the determining factors.[4,7-12] From the nineties onwards, research has mainly focused on the effect of GH treatment in children with PWS, and these studies have revealed that GH positively influences body composition, but it does not normalize these features.[13] There are also some studies that indicate that GH treatment improves motor development in infants.[6,9,14]

The aim of this thesis was threefold: first, to contribute towards a better understanding of motor development in infants with PWS; second, to gain insight into the effect of child-specific physical training combined with GH treatment on muscle strength, muscle mass, and motor development; and, third, to increase the quality of care for these infants and their parents by developing a child-specific intervention program.

This chapter starts with a description of the syndrome, followed by an explanation of the theoretical perspective on (motor) development used in this thesis. After this, a case report is provided to illustrate the motor developmental problems that PWS infants encounter in different developmental stages, combined with the questions and concerns that parents have in caregiving. Finally, the research questions and outline of the thesis are presented.

Prader-Willi syndrome

PWS results from lack of expression of a paternally imprinted region of chromosome 15q11-13. In about 70% of the cases, it is caused by a paternal deletion;[15,16] in about 25%, it is caused by maternal uniparental disomy;[17-19] and in 1–5%, it is caused by an imprinting center mutation or translocation.[20,21] PWS is characterized by a wide variety of physical, cognitive, and behavioral

defects. The symptom presentation is mostly age-dependent, not all symptoms are expressed in all patients, and the severity of disabilities differs between patients.

In infancy, muscular hypotonia, poor sucking reflexes, feeding problems, failure to thrive, and psychomotor delays are most prominent.[3] A majority of the newborns is severely hypotonic, less active, and sometimes almost motionless.[4] After weeks or months, the infants become more responsive and are able to show more spontaneous movements, although they continue to suffer from hypotonia and muscle weakness. As a result, motor development is seriously delayed.[4-6, 22]

In childhood, hyperphagia as well as cognitive, social, and behavioral deficits become more prominent.[3] In adulthood, there is a lack of complete pubertal development and cognitive, emotional, and psychiatric problems are common.[3] Many of the symptoms in PWS are thought to be the result of hypothalamic dysfunction.

Hypothalamic dysfunction

There are some indications for hypothalamic dysfunction in PWS patients. Firstly, several neurological abnormalities in the structure and function of the hypothalamus have been reported: hypoplasia of the pituitary gland;[23] size reduction or absence of the posterior pituitary bright spot;[24,25] reduced thionine-stained volume and cell number of the paraventricular nucleus;[26] and diminished vasopressin expression.[27] Secondly, the hypothalamus is involved in regulating the endocrine and autonomic nervous systems, both of which are affected in individuals with PWS. Concerning hormonal functioning, regulation of the thyroid is affected in PWS patients; free T4 levels are low, and basal metabolic rate and activity levels are reduced.[28,29] The regulation of gonads is affected, and in infancy and childhood, hypogenitalism, cryptorchidism, and hypogonadism are common,[30,31] with incomplete or absent pubertal development and infertility.[30,32] Central adrenal insufficiency is reported in PWS patients.[33] Furthermore, in PWS patients, GH secretion is insufficient.[34] They have a short stature, reduced muscle mass, increased fat mass, and reduced bone mineral density.[35] Concerning the autonomic nervous system, the following symptoms are reported: hypoventilation during sleep, sleep apnea, abnormal awake ventilator responses to hypoxia and hypercapnia, reduced pulmonary function, abnormal temperature regulation, abnormal Rapid eye movement (REM) sleep, and excessive daytime sleepiness.[36-39] Moreover, there are feeding difficulties in infancy.[40,41] In contrast, in childhood and adulthood hyperphagia develops, mechanisms of satiety are impaired, and the patient is unable to vomit.[3,17,42]

Developmental profile in PWS

In PWS infancy, psychomotor development is severely affected by hypotonia and muscle weakness.[4-6] In these young PWS infants, gross motor development, eye-hand coordination, and speech are more affected than social development and intelligence.[5,22,43] In childhood, hyperphagia, mood fluctuations, temper tantrums, stubbornness, and ritualistic, repetitive, perseverative, obsessive, and/or compulsive behaviors, including skin-picking develop.[44-46]

Hyperphagia is usually combined with preoccupations for all sorts of food, food seeking, and eating unusual food-related items.[44] The maladaptive behaviors appear to escalate with age, increasing in severity and intensity and are not reducing in adulthood.[44-46] Most PWS adults function in the "mild retardation range" and have problems with attention, short-term memory, auditory processing, and sequential processing.[45,47,48] Social functioning and the ability to interpret social information is poor, even considering their reduced intellectual abilities.[49] Psychiatric problems are highly prevalent in PWS adults and mostly caused by depressive illness with psychotic symptoms.[50]

A dynamic systems approach to (motor) development

The physical therapy intervention used in this thesis is based on a dynamic systems perspective on (motor) development. In this perspective, developmental changes are the result of a complex interaction between the child, its environment, and goal-directed actions or tasks. None of these three interacting factors is dominant and changes in behavior are nonlinear and emergent.[51,52] In the intervention, an infant is challenged to actively explore and exploit the biomechanical properties of its body in relation to its environment to use it in a functional manner, in a goal-directed action.[53,54] The dynamical systems theory contrasts greatly with the early maturational theories in which (motor) development was viewed as an autonomous biologically driven process. Motor developmental change was a direct read-out of the maturational status of the nervous system, in which the influences of the environment, therapy, or practice was of minor importance.[51,52]

From the 1920s, scientists started to study motor development from a maturational perspective. The classic descriptive studies of the sequence of motor milestones (roll, crawl, sit, stand, and walk) from that time (e.g., Gesell,[55] and McGraw,[56]) form the basis for standardized motor developmental tests widely used in the pediatric clinical practice today. However, although these motor milestones are relatively invariant between infants and cultures, the order and timing in which children learn these skills differ and the quality of movement patterns differ.[57] In the early eighties, inspired by the dynamical systems theory, Esther Thelen did some ingenious experiments to study newborn stepping (stepping reflex). As can be observed in clinical practice, newborn infants make stepping movements when held upright immediately after birth, which disappear a few weeks later. The explanation from a maturational viewpoint, was that this "primitive" reflex was inhibited during the maturation of cortical centers.[58] It was found that infants in which newborn stepping had disappeared performed kicking movements in supine position that were kinematically similar to the early inborn stepping movements.[58] Also, when these infants were held upright in water, the stepping pattern reemerged.[59] Moreover, when small weights were added to the legs of infants that still showed newborn stepping, there was a decrease in the frequency and flexion of the legs.[59] These experiments demonstrated that task performance (stepping) was influenced by the existing interaction between growth and changing body characteristics and the environment, especially in relation to gravity. The disappearance of

newborn stepping could be explained as a consequence of postnatal rapid weight gain, in which infants acquire fat at a greater rate than muscle mass, which leads to relatively less muscle force and an environment-related incapacity to perform the task.[58,59] The experiments of Thelen were the starting point of a major intellectual shift in the view on motor development and the designing of rich learning environments.

Remarkable discoveries in neuroscience about brain organization, brain development, and plasticity have shaped the way we think about development in general. First, based on the anatomic structural organization and interconnectivity of brain areas, perception, action, and cognition are found to be part of the same continuous and coupled process.[51] Second, the development of the human brain is a long-lasting dynamic process in interaction with the environment. In the prenatal phase, neurons already start to interact by the formation of dendrites, axons, synapses, and the production of neurotransmitters. The process of neurogenesis is particularly active in the few months before birth till the end of the first postnatal year.[60,61] However, synapse formation and neurotransmitter production continue throughout life.[60,62] Also, axonal myelination starts in the prenatal phase and grows rapidly during the first few years of life, but continues to grow until the age of 30 years.[60,63] A remarkable feature of brain development is that it consists not only of the creation of components but also of their elimination. Approximately half of the created neurons die off before birth; axon retraction is present till about 1 year after birth; and synapses are eliminated especially between 18 months of age and the onset of puberty.[60,62] Third, the brain is remarkably dynamic and plastic. Experience and interaction with the environment shape the nervous system.[51,52] Neural elements that fit the environment best persist, thus allowing an adaptation of the brain to its environment.[62] Hence, the functional mapping of the brain is experience-dependent, especially through perceptual-motor exploration.[51,62]

Stimulating early development is of high importance, because the first 2 years of life are especially critical for brain development (sensitive periods). From a dynamic systems perspective, interventions should be focused on manipulating the task and environment in such a way that the child becomes able to explore the biomechanical properties of its body and simultaneously learns how to use its constrained (motor) abilities in a functional manner. New learned skills are added to the actual motor repertoire and will be the basis for the next transition.[53] From this perspective, hypotonia and low muscle strength in PWS infants are important constraints in overcoming gravity and repeating movements with sufficient frequency to achieve a learning effect. This results not only in severely delayed motor milestones but also in structural changes in the musculoskeletal and neurological systems.

Muscle strength training

The muscle strength-generating capacity of skeletal muscles is practically equal per muscle fiber surface in all mammals.[64] Therefore, muscle strength increases normally with the increase of muscle mass by growth as measured by height and weight. Moreover, neuronal factors such as

the increase and activation of motor units, changes in the coordination of these motor units, recruitment, and signal excitement are also important determinants of muscle force increment in infants.[65-67] However, the exact mechanisms underlying muscle force increment during development are not yet clear.

In muscle strength training, a progressive resistance is normally used.[68] Different research results show that typically developing children can increase their muscle strength by 30–40% by short resistance training programs; however, no results regarding infants are available.[68-70] One of the most striking findings in the response to strength training in children compared with adults is that in children, the increased muscle strength is not related to an increase in muscle mass (hypertrophia),[65,66] although one study found a trend.[71] It is hypothesized that this reduced muscle mass increment is the effect of an absence of testosterone. We hypothesized in this infant study that GH would be responsible for the increment in muscle mass if muscles were trained at the same time.

One of the most frequently used models to calculate the starting level for muscle strength training is the Repetition Maximum (RM) model.[68,69] This model allows for functional muscle strength training and is therefore usable to train muscle force in infants who are not able to perform resistance exercises. The training intensity can be determined by the repetition level and 8–15 repetitions are recommended.[68,69] In our infant training program, we used a repetition level of 10 repetitions as optimal. This was because this number of repetitions allows skill training combined with a training stimulus to increase muscle strength.

Motor development in PWS: a clinical case report

In the present thesis, studies in 22 infants with PWS are described. We followed these infants during two years, and their motor developmental progress was evaluated every three months. Never before was motor development in PWS infants studied so extensively. In the case report (Boxes 1–4), an overview of the first years of life for one of the 22 infants (Noor) is presented. I chose Noor because she was relatively young at the first assessment, and she was able to reach all motor milestones until the ability to walk independently during the time span of the present study. In this case report, the motor developmental problems these infants encountered in daily life and the challenges for their parents in caregiving are illustrated together with some elements from the intervention program.

Taken together, it is obvious that parents need to deal with several challenges. In the first years, motor development is severely affected and is a central point in caregiving. We learned a lot from the participating infants and parents and from the researchers before us. The results of these clinical experiences and studies are presented in this thesis.

Box 1: The first six months of Noor's life

Noor was born in a general hospital after a full-term pregnancy. Due to serious feeding difficulties, severe hypotonia, and poor movement ability, she stayed at the hospital in the first weeks of her life. A few weeks after birth, genetic counseling confirmed the diagnosis Prader-Willi syndrome (PWS).

We saw Noor for the first time at the age of 5.5 months. At that moment, she was in good health and the main issues for her parents were feeding difficulties, concerns about hip dysplasia, and uncertainty about handling and caregiving.

Noor's parents felt unsure whether Noor was eating enough, because Noor did not announce herself. Moreover, her parents read about overeating and the inability to vomit as characteristic of PWS, so they were very insecure about feeding. We informed the parents that at this young age, overeating does not occur in PWS and advised them to follow the volume in the prescribed feeding scheme from the pediatrician. Because bottle feeding took a long time, it was advised to avoid fatigue, and stimulate sucking by starting each feeding session with bottle feeding for a maximum of 15–20 minutes. The leftover should be given by tube. We showed Noor's parents how to support Noor during feeding and how to stabilize the position in the maxi-cosi during transport in a car or stroller using a "polka dot pillow". More instructions were given for bathing, picking up, and carrying.

Noor's parents reported being very concerned about Noor's hips, because they heard a knocking sound and felt a crack when she positioned her legs to a frog-like posture. This was verified during the physical examination, and we noticed asymmetry in active leg movements. Although ultrasound scanning of both hips had already been performed and was normal, we advised consulting the orthopedic physician for a reevaluation, which confirmed hip dysplasia.

Noor was severely hypotonic and struggled to move against gravity in supine and prone positions. Although she was able to turn her head, lift her lower arms to reach for a toy, and roll to her side, she could not play with toys in the supine position and repeated movements only one or two times, which illustrated low muscle strength, and was not enough to learn new skills adequately. She could move her legs, but she was not able to lift them. Lying prone, she was able to lift her head only for a few seconds. Overall, Noor had trouble controlling her posture, her movements were much slower than in typically developing infants, and she was easily fatigued. We demonstrated how the parents could diminish the negative influence of gravitational forces on Noor's body in different positions, for example, by supporting Noor's posture in supine using a "polka dot pillow" to support arms and legs in adduction, and to use a special triangle cushion to realize a more vertical well-supported position. Parents noted immediately the increased number of repeated movements essential to learning new skills.

Box 2: Noor around her first birthday

From eight months of age, tube feeding was no longer needed and Noor started babbling. Noor was eating an adequate volume by bottle. A specialized speech therapist was consulted to guide the transition from liquid food to solid food and Noor was learning to eat using her hands. During eating, she needed a support to be able to sit during the meals.

During a period of six months of immobilization by hip plaster because of hip dysplasia, Noor's ability to move was radically constrained. At 11 months of age, hip immobilization was finished. Noor was not able to crawl on her knees, which slipped outwards in a frog-like position as a result of hypotonia, low muscle strength, and a deficiency in hip control. We instructed the parents to exercise to improve hip muscle strength and control. For example, the crawling posture was practiced while Noor's belly or knees were supported just enough to gain strength and control. When Noor was able to maintain a crawling posture, a towel was placed around her belly to support her trunk to enable her to learn to crawl.

A few months later, we could tell the parents that compared with other PWS infants, Noor developed well. She had a motor developmental level of about 70% of typically developing infants, whereas the average in PWS is 55%. Noor was able to sit stably, crawl on her belly, rise to a sit, and she was able to reach, grasp, and look behind sitting on the floor. However, she used an abnormal strategy to come from prone lying to sitting: she pushed herself up with her arms while her legs went from a frog-like position to a sideward spread leg position, and when she was sitting on her buttocks, she moved her legs forward. This strategy is seldom observed in typically developing infants, but is often seen in infants with hypotonia and hyperlaxia (e.g., Down syndrome).

Box 3: Noor at two years of age

Noor had an ear infection and high fever for the first time, and she needed antibiotics to recover. Parents already indicated that she was not well a week before. Hypotonia increased, she was really tired and weary, and lied down during the day to fall asleep for short moments. Fever is not a clear indicator for sickness in PWS; a patient can be severely ill without having fever. Therefore, parents of children with PWS are instructed to contact their pediatrician when they notice signs that their child is sick.

At 18 months of age, Noor used more and more different sounds and expressed herself with pointing and nonverbal signs. She was able to understand and accomplish small orders, but active use of words was very limited. After starting speech therapy, she spoke her first words within a few weeks and used sign language to communicate. Recommendation to start speech therapy is common in PWS around the age of two years.

Noor was able to stand without support at the age of 24 months for a short moment. She was able to stand up by pulling herself up and during the assessment, Noor made her first two steps independently (normally between 10–13 months). The recommendations were focused on standing and walking independently in a playful way: for example, by giving her a large but light object for which she needed both hands. For walking, we showed that it helped Noor when she was supported firmly until she stood stably and then let go to the other parent for a great hug.

In this period, Noor developed a very strong will, and tended to have temper tantrum outbursts when things did not go her way. Her parents were struggling with the question of whether this was normal toddler behavior or whether this was a PWS characteristic. It was presumably a little of both. In Noor's case, emotional development seemed only mildly affected, because the parents could distract her very easily and she did not show ritualistic or obsessive behavior.

Box 4: Noor as a toddler

Although Noor's hips were normalized at 32 months of age, it appeared that the hip socket was not fully formed at the right side and there was a small risk for dislocation. Therefore, a focus on muscle strength training for the muscles around the hip was important, which was discussed with Noor's pediatric physical therapist.

Noor had a strong will and she could become very angry. With the concepts of "naughty" and "sweet", she could be corrected and Noor's parents were still capable to cope with Noor's strong will. Noor did not show obsessive behaviors about food. She was able to express herself more in spoken language and imitative playing, and she was able to perform fine motor skills, such as threading beans; however, drawing figures was difficult. During the assessments, Noor showed skills at a higher cognitive developmental level than average in PWS, which was confirmed by her scores on a cognitive developmental test.

Noor's walking abilities extended: she was able to walk across a small line, forwards and backwards, and stepped up and down. She was also able to increase her walking speed but was not able to run, and when she wanted to jump, she initiated the movement but could not lift her feet from the floor. She was able to cycle on a tricycle, and was very proud that she could stand on one leg for a moment. However, it was obvious that her peers were far ahead of her. Still, hypotonia and muscle strength were low, visible in her severe lordosis and overstretched knees while standing.

Because this was the last visit, the recommendations were more general and focused on the future. It was recommended to start endurance training to prepare Noor for further motor milestones and joyful participation in sport and games with her peers.

Research questions

The aim of this thesis was to increase understanding of motor development in PWS infants and the effect of physical training combined with GH treatment on the motor developmental process. Five main research questions were defined:

Research questions:

1. What is reported in the literature about motor problems in PWS, their underlying causes, and the effect of interventions on motor performance in PWS?
2. Which characteristics are typical in motor development for infants with PWS?
3. What is the effect of standardized child-specific physical training in combination with GH treatment on motor development?
4. Can muscle strength be objectively measured in normally developing infants and in infants with PWS?
5. What is the relationship between GH treatment, muscle mass, muscle strength, and motor development in PWS?

Although motor problems are characteristic in PWS infancy, studies focusing on motor development are scarce. Therefore, our first study aimed to provide an overview of the current state of the art and we performed an extensive systematic literature search to find all reports on motor performance in PWS, the underlying causes of the motor problems, and the effects of

physical training and GH treatment on motor performance in PWS. The results of this search were incorporated in two systematic reviews: one about body composition and neuromuscular functioning in PWS (Chapter 2), and one about motor performance in PWS and the effect of physical training and GH treatment on motor performance (Chapter 3).

We hypothesized that GH could enhance the effect of physical training on motor development in PWS infants. Moreover, it was obvious that such an intervention should be infant-centered and guided by the questions for help from the parents. Therefore, we developed a standardized intervention that could be performed by a pediatric physical therapist in the neighborhood of the family, guided by a therapist of our expertise center (Radboud university medical center). The program focused on supporting the child with antigravity movements, training muscle strength, and stimulating skill learning. We studied the effects of adding GH treatment to the standardized child-specific physical training on motor development in PWS infants, in a longitudinal randomized clinical trial. For each child, we evaluated motor development every three months over two years. The results of this study are presented in Chapter 4.
When we designed the longitudinal study, it was not possible to quantify muscle strength in infants objectively, because no measurement instrument was available. Techniques to assess muscle strength in children and adults were well established and validated.[72-74] Unfortunately, none of these methods were suitable for infants. Therefore, we developed a new measurement method to quantify muscle strength in infants and toddlers, the “Infant Muscle Strength meter” (IMS-meter). We tested the IMS-meter in typically developing infants between six and 36 months of age and compared the outcome with PWS infants of 24 months of age. We determined the reliability of the IMS-meter, gathered reference data, and developed a model that predicted muscle strength in typically developing infants. The results are presented in Chapter 5. In Chapter 6, we tested the effect of training with and without GH on muscle mass and the relationship with muscle strength and motor performance in PWS infants. In the general discussion (Chapter 7), gaps, new ideas, and findings in clinical thinking, and practice and research on motor problems in PWS are described. Chapters 8 and 9 provide a summary of this thesis in English and Dutch respectively.

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2

Motor problems in Prader–Willisyndrome: A systematic review on body composition and neuromuscular functioning

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Abstract

Motor problems in Prader–Willi syndrome (PWS) are presumably related to abnormal body composition and certain neuromuscular abnormalities. The authors reviewed the literature to evaluate the extent to which body composition is affected and gathered all findings on neuromuscular functioning in PWS. A systematic review was conducted in four databases (1956–2010). The methodological quality of each included article was evaluated. Thirty-eight papers were included: body composition (9 studies), neuromuscular functioning (7) and growth hormone (GH) effect studies (23). Increased fat mass and decreased lean body mass are characteristics of PWS. As a result, muscle mass is decreased by 25–37%, which might explain partly the weakness and hypotonia. However, there are also structural and functional muscle abnormalities, and cortical motor areas are hypo-excitable in PWS patients. Moreover, disuse as result of decreased activity in PWS could also contribute. GH treatment positively influences body composition, but does not normalize it. Training could prevent disuse and improves body composition. Therefore GH treatment and training will probably enhance one another.

Introduction

Prader, Labhart, and Willi first described Prader–Willisyndrome (PWS) in 1956[1] PWS is a neurogenetic disorder resulting from absent expression of the paternal region of chromosome 15q11-13. In about 70% of cases it is caused by a paternal deletion,[2,3] in about 25% of cases by maternal uniparental disomy,[4-6] and in 1–5% it is caused by an imprinting center mutation or translocation.[7,8] The estimated prevalence is 1 in 10,000–30,000 births.[9] PWS is characterized by a wide variety of physical, cognitive, and behavioural defects. The most significant characteristics are hypotonia in infancy, hypogonadism, obesity, short stature, motordelay, cognitive deficits, and mild dysmorphic facial features.[4,10] The presence of symptoms is mostly age-dependent; not all symptoms are expressed in all patients, and the severity of disabilities differs between patients. In this paper we are particularly interested in the symptoms in PWS which contribute to their motor problems.

Motor performance in PWS is particularly affected in infancy. In the majority of cases, newborns are severely hypotonic, inactive and sometimes almost motionless. After several weeks or months the infants become more responsive and are capable of more spontaneous movement, although they continue to suffer from hypotonia, muscle weakness and as a result severely delayed motor development.[10-14] Motor problems are still present in childhood and adulthood, and decreased activity in PWS patients is reported.[15-19] PWS patients score well below the normal range on standardized motor performance tests,[20,21] and they have an abnormal gait pattern.[22] The causes of hypotonia, muscle weakness, and motor problems in PWS patients are not clear; it is presumed however that the contributing factors are the abnormal body composition in PWS patients, with an increase in fat mass and a decrease in muscle mass, and possibly some degree of neuromuscular abnormality.[23,24]

Abnormal body composition in PWS patients is thought to be related to hormonal deficiencies as a result of hypothalamic dysfunction.[25-27] Indeed several studies have found anatomical and functional abnormalities of the hypothalamus.[28-32] Moreover, growth hormone (GH)[33] and hormones of the thyroid,[15,34] gonads,[35,36] and adrenal cortex[37] are found to be affected. The most significant finding in relation to body composition in PWS patients is GH deficiency. GH deficiency presumably leads to the short stature, decreased lean body mass, increased fat mass, and decreased bone mineral density in PWS patients.[33] GH treatment leads not only to an increase in height, but also to a decrease in fat mass and an increase in lean body mass in PWS patients.[20,38-40] In some GH studies it is presumed that GH treatment could also have a positive effect on muscle strength, motor development and/or performance, because GH positively influences body composition.[38,39,41]

To evaluate the extent to which the body composition abnormalities in PWS may contribute to the motor problems, it is necessary to gather insight into the severity of the abnormalities in relation to age, as well as their progress over time. To our knowledge there are no longitudinal studies that focus on body composition in PWS. There are several cross-sectional studies but the results of these studies have never been systematically reviewed. We wanted to perform a

systematical literature study on body composition in PWS to critically review the findings and obtain more insight into the presumed relationship between body composition abnormalities, hypotonia, muscle weakness, and motor problems in PWS. Additionally it has been hypothesized that abnormalities in neuromuscular functioning could contribute to the motor problems in PWS patients. Only three studies are frequently mentioned with regard to this hypothesis.[11,24,42] We wanted to know if there are more studies that focus on neuromuscular functioning in PWS and therefore expanded our literature study to both topics. In this paper we describe the findings of our systematic literature study regarding body composition and neuromuscular functioning in PWS and review the effects of GH treatment on body composition. The methodological quality of all references included in this review was assessed in order to evaluate the quality and authority of the reported findings.

Method

Search strategy

We identified relevant papers in a comprehensive literature search that focused on body composition and neuromuscular functioning in PWS. First of all, a search for English-language publications was conducted, covering the period from January 1956, the year in which the first description of the syndrome was published, to January 2010 and using four electronic databases: Pubmed, Embase, Cinahl, and Psycinfo. Since only Embase and Psycinfo used the same thesaurus terms and major subheadings, the searches were performed separately in each database and results were combined afterwards. The articles on body composition in PWS were found based on “bodycomposition” as search and meshterm. The articles on neuromuscular functioning were found using the following search or meshterms: muscle(s), musculoskeletal, neural physiological phenomena, musculoskeletal system, musculoskeletal development, and musculoskeletal physiological phenomena. The searchstrings are listed in Appendix A. The reference lists from relevant papers were then checked manually for supplementary articles not previously identified.

Selection criteria

Overlapping articles were deleted. Each abstract was screened by two authors (L.R. and M.N.) and included if it was related to an original scientific article focusing on either body composition orn euromuscular functioning in PWS. The article was excluded if it concerned a casereport, an opinion-based article, a non-systematical review, or animal research and if there was no abstract available. Furthermore, articles were excluded if the main subject was outside the scope of our study. The exclusion criteria are presented in Table 1. After selection the full text article was screened again based on the inclusion and exclusion criteria (Figure 1).

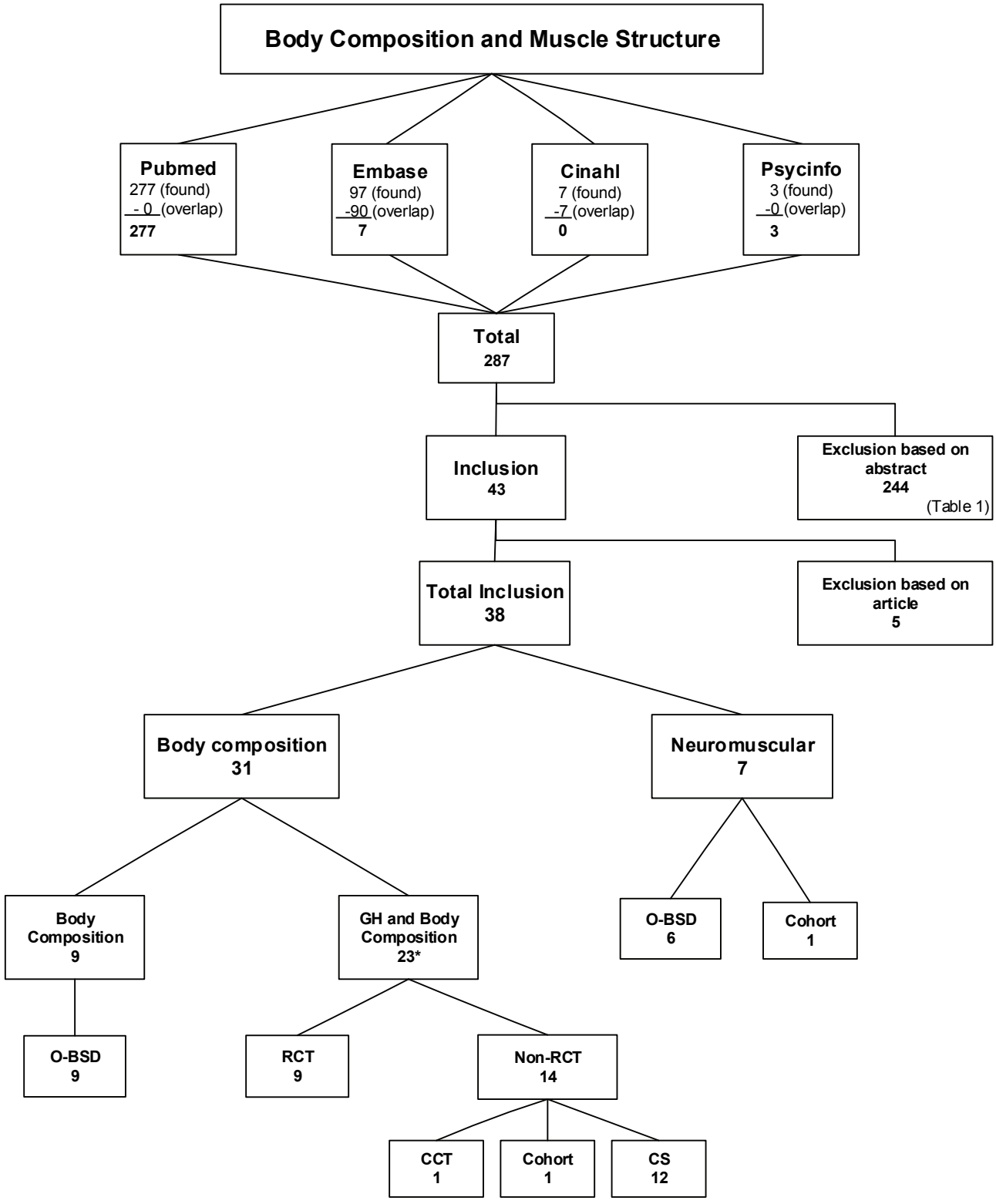

Figure 1. Flow chart of selection procedure

GH = Growth Hormone, O-BSD = Observational Between-Subject Design, RCT = Randomized Controlled Trial, CCT = Clinical Controlled Trial, Cohort = Cohort Design, CS = Case Series

*The literature search revealed 22 references. In total 23 studies were evaluated, since one reference contained results from an RCT as well as a CS.

Table 1. Exclusion criteria

Reason for exclusion	Number of articles excluded
Case report	15
Non-systematical review/Opinion-based	12
Animal research	5
No abstract available	8
Syndrome different from PWS/not PWS specific	32
Different focus:	
Breathing/sleep	31
Behavior/cognition/language	15
Glucose metabolism/insulin/diabetic	15
Hyperphagia/diet/obesity	15
Fitness	14
Bone mineral density/scoliosis/hip dysplasia/orthopedic	14
Genetic	12
Hormonal functioning	10
Visual perception	7
Anthropometric/craniofacial features	6
Cardiovascular disease	5
Treatment guidance	5
Other	23
	244 Total

Evaluation of the methodological quality

The methodological quality of the included references was evaluated based on technical aspects of the study design. After excluding "Casereports" and "Non-systematical reviews", we included five different study designs: Randomized Controlled Trials (RCT), Cohort Designs, Clinical Controlled Trials (CCT), Case Series (CS), and Observational Between-Subject Designs (O-BSD). Since standard checklists have not been developed for all of these designs, we therefore selected criteria from three standard checklists: Evidence-Based RichtlijnOntwikkeling (EBRO)[43], the Physiotherapy Evidence Database Scale (PEDro)[44] and the Critical Appraisal Skills Programme (CASP)[45]. We selected thirteen relevant criteria: (1) Randomization, (2) Group definition, (3) Selection bias, (4) Group comparability, (5) Treatment procedure, (6) Blinding, (7) Control period, (8) Study duration, (9) Measurement procedure, (10) Comparability of treatment, (11) Confounding effects, (12) Loss, and (13) Intention-to-treat. All 13 criteria were used to evaluate the RCT, for the CCT 12, for the Cohorts 10, for the CS 7, and for the O-BSD 6. Two authors (L.R. and L.V.) independently scored each criterion. Its quality was determined once it was established whether or not the criterion was reported. If the criterion was well described and its quality was good, two points were assigned for that criterion. One point was assigned if the criterion was incompletely described and/or its quality was doubtful. Zero points were assigned if the criterion was not described. The level of agreement between authors was determined by Cohen's Kappa. The percentage of the maximum score was calculated in order to compare the evaluations of

different designs. For example: the maximum score for an RCT was 26 points (13×2 points); if a study was evaluated with 18 points, it scored 69%.

Results

Results of electronic database search

The electronic search identified 287 unique references, 38 of which met our inclusion criteria. A flow chart of the selection procedure is presented in Figure 1. Thirty-one references were found on body composition in PWS, of which nine were solely focused on body composition and 22 on the effects of GH treatment on body composition. In total 23 GH effect studies were evaluated, since one study contained results from an RCT as well as a CS. Seven references were found on neuromuscular functioning in PWS. The body composition studies (n=9) were all O-BSD. Six of the neuromuscular studies were O-BSD and there was one Cohort. In the GH effect studies four design types were found: RCT (n=9), CCT (n=1), Cohort (n=1), and CS (n=12).

Body composition in PWS

Study characteristics

A description of the study characteristics is presented in Table 2. All nine body composition references were O-BSD. Body composition was compared to normal healthy individuals (n=4) or obese individuals (n=6). In six studies the PWS group was smaller than 20 patients and in three studies the group was larger than 20 patients.

Methodological quality

Initially the raters reached an agreement of 87% (Cohen's Kappa 0.77) with respect to all the criteria of the nine O-BSD studies (9 × 6 = 54 criteria). 100% consensus was reached following consultation. The quality scores for the articles reached 67 and 82% of the maximum score. The selection procedure of patients is not described in any of the O-BSD studies, but from the patient descriptions it is possible to evaluate whether or not the patients included are representative of PWS patients in general. Seven studies do not report whether there was patient loss. In the three studies with the lowest scores (67%), authors do not correct for possible differences in height between control subjects and PWS patients, who are normally significantly shorter.

Methods used

Body composition can be measured using different techniques based on different principles and assumptions. Dual Energy X-Ray Absorptiometry (DEXA) is a scanning technique that measures the differential attenuation of two X-rays as they pass through the body. It distinguishes total Bone Mineral Content (BMC) from soft tissue and subsequently divides the latter into fat and lean tissue. Lean tissue consists mainly of muscle tissue and is called Lean Body Mass (LBM). Fat Free Mass (FFM) is the sum of LBM and BMC.[46] Another way of measuring body composition is based on total body water, which is called Deuterium Dilution (DD). First total body water is

Table 2. Characteristics of body composition studies

ID	Study	Sample	Measurements
2.1	Bekx et al., 2003 [50]	**Infants** - 16 PWS (m = 12, 5-23 mo) - Norm values.	H; W; BMI; BF%, FFM (*DEXA*); BF%, FFM (*DD, n=14*); TEE (*DLW, n=14*); REE (*IC*)
2.2	Eiholzer et al., 1999 [51]	**Infants-Children** - 13 PWS under-W (m = 1, 0-4 y) - 10 PWS over-W (m = 6, 1-10 y) - Norm values	H; W; W/H; BMI; arm-C; Skinfolds; Leptin (*n=22*); BF% (*DEXA, n=8*)
2.3	Hill et al., 1990 [17]	**Children** - 36 PWS (m = 13 y) - 20 N-control (m = 10 y) - 11 O-control (m = 10 y)	H; W; BF%, FFM (*BIA*); Skinfolds; RMR (*IC*)
2.4	Van Mil et al., 2000 [19]	**Children-Adults** - 17 PWS (m = 12, 7-20 y) - 17 O-control	H; W; BMI; FFM, BF%, ADMR (*DLW*); BMR (*IC*); AEE
2.5	Brambilla et al., 1997 [48]	**Children-Adults** - 27 PWS (m = ?, 6-22 y) - 27 N-control - 27 O-control	W; H; BMI; W/H; BF%, BF%-limbs, BF%-trunk, LBM, LBM-limbs, LBM-trunk, BMC (*DEXA*)
2.6	Van Mil et al., 2000 [52]	**Children-Adults** - 17 PWS (m = 12, 7-20 y) - 17 O-control (m = 11 y)	H; W; BMI; SMR (*respiratory chamber*); BMR (*IC*); FFM, FFMI, BF%, BFI (*DD*)
2.7	Van Mil et al., 2001 [53] *Suppl. Van Mil et al., 2000* [52]	**Children-Adults** - 17 PWS (m = 12, 7-20 y) - 17 O-control (m = 11 y)	H; W; BMI; BMR (*IC*); TEE (*DLW*); AEE; FFM, FFMI, BF%, BFI (*DD*); BF%, BMC (*DEXA*)
2.8	Theodoro et al., 2006 [49]	**Children-Adults** - 48 PWS (m = 23, 10-45 y) - 24 O-control (m = 26, 11-49 y)	H; W; waist-C; hip-C; BMI; BF%, BF%-limbs, BF%-trunk, FFM, FFM-limbs, FFM-trunk, BMD (*DEXA*)
2.9	Schoeller et al., 1988 [18]	**Children-Adults** - 10 PWS (m = ?, 8-24 y) - 10 O-control	H; W; BMI; BF%, FFM (*DD*); TEE (*DLW*); BMR (*IC*); TDEE-equation; AEE (*TEE and TDEE-equation was used to calculate AEE*)

Sample: m = mean age and age range, N-control = normal control group, O-control = obese control group. **Anthropometric measurements**: H = height, W = weight, BMC = bone mineral content, BMI = body mass index, Arm-C = arm circumference, Waist-C = waist circumference, Hip-C = hip circumference. **Body composition measurements**: BF% = body fat percentage, BF%-limbs = body fat percentage of limbs, BF%-trunk = body fat percentage of trunk, BFI = body fat index, FFM = fat free mass, FFMI = fat free mass index, FFM-trunk = fat free mass of trunk, FFM-limbs = fat free mass of limbs, LBM = lean body mass, LBM-limbs = LBM of limbs, LBM-trunk = LBM of trunk, Leptin = leptin levels. **Energy measurements:** TEE = total energy expenditure, AEE = energy expenditure during activity, TDEE-equation = total daily energy expenditure based on equation, ADMR = average daily metabolic rate, BMR = basal metabolic rate, RMR = resting metabolic rate, SMR = sleeping metabolic rate, AEE = activity related energy expenditure ((0.9 x ADMR)-BMR). **Measurement methods:** DD = deuterium dilution, DLW = double labeled water, DEXA = dual energy X-ray absorptiometry, IC = indirect calorimetry, BIA = bioelectrical impedance analysis. **Effect indication:** = = similar, ≠ = different, ↑ = increased, ↓ = decreased, + = positive, (X * Y) = correlation between X and Y.

Main findings	Conclusions	Quality
PWS vs. Norm values: H ↓, W ↓, BF% ↑, FFM ↓, TEE ↓, TEE/FFM =, (TEE * FFM) =	Even in underweight PWS infants BF% is increased. Lower TEE in infants with PWS is caused by decreased FFM.	67%
PWS under-W vs. Norm: Skinfolds =, arm-C ↓, Leptin ↑, Skinfolds/BMI ↑ **PWS over-W vs. Norm:** Skinfolds ↑, Leptin ↑, Skinfolds/BMI ↑	Body composition in PWS is already abnormal in infancy, before the onset of obesity.	82%
PWS vs. Norm: (W * FFM) ≠, RMR ↓, (RMR * W) ≠, (RMR * FFM) ≠ **PWS vs. O-control:** RMR ↓, (RMR * W) ≠, (RMR * FFM) ≠ **O-control vs. N-control:** RMR ↑, (RMR * W) =, (RMR * FFM) =	Reduced FFM is not the sole explanation for the lower energy expenditure seen in patients with PWS.	67%
PWS vs. O-control: H =, W =, BMI =, BF% =, FFM ↓, BMR ↓, ADMR ↓, BMR/FFM =, BMR/W ↓, ADMR/W ↓, ADMR/FFM ↓	AEE is decreased in PWS which implies decreased physical activity.	75%
PWS vs. N-control: BF% ↑, LBM ↓, LBM-limbs ↓, LBM-trunk ↓, FM/LBM ↑ **PWS vs. O-control:** BF% ↑, BF%-limbs ↑, BF%-trunk =, LBM ↓, FM/LBM ↑ **O-control vs. N-control:** BF% ↑, LBM ↑, BMC ↑	PWS patients show a peculiar body composition, to some extent similar to that found in subjects deficient in growth hormone.	75%
PWS vs. O-control: FFM ↓, FFMI ↓, FM =, FMI =, SMR ↓, BMR ↓, (SMR * FFM) =, (BMR * FFM) =	BMR and SMR are low in patients with PWS because of a low FFM.	75%
PWS vs. O-control: FFM ↓, FFMI ↓, FM =, FMI =, (FMI * FFMI) =, (FMI * FFMI) ↓	In PWS adiposity is probably related to abnormal GH function and physical inactivity.	75%
PWS vs. O-control: H ↓, W ↓, BMI ↓, LBM/H ↓, LBM-limbs ↓, LBM-trunk ↓, FM/LBM ↑	Body composition in PWS is characterized by reduced LBM in both limbs and trunk and increased BF%.	75%
PWS vs. O-control: H ↓, W ↓, BF% =, LBM ↓, TEE ↓, AEE/TDEE ↓ **O-control:** AEE = 50% of TEE (18.3 kcal/kg/d) **PWS:** AEE = 35% of TEE (11.5 kcal/kg/d)	TEE is lower in PWS patients, because of decreased LBM and reduced physical activity.	67%

measured, usually with the double labeled water. Subsequently FFM is computed based on the assumption that total body water represents a fixed proportion of FFM (hydration factor of 0.73) and that fat is anhydrous. Fat mass is calculated by subtracting the FFM from the total body weight.[46] Since FFM and body fat have different electrical properties it is also possible to measure body composition with Bioelectrical Impedance Analysis (BIA). In BIA two conductors are applied to the body and resistance is measured. Conductivity through FFM, which consists of water and electrolytes, is greater than through body fat. Based on the resistance, and taking into account gender, weight, and height, it is possible to calculate fat mass and FFM.[47]

Both DEXA and DD are accurate methods for measuring body composition.[46] DEXA has the advantage that it not only distinguishes fat mass and FFM, but also LBM and BMC; in addition it can define body composition in different body regions.[48,49] BIA is a less accurate technique for

measuring body composition; it is generally assumed that it requires pathology-specific equations, which are not available for PWS.[46]

Results of body composition in PWS

The results of the nine body composition articles were added to the results of six GH studies in which PWS patients were compared to norm values before the start of GH treatment (Table 4: 4.1, 4.2, 4.3, 4.5, and Table 5: 5.1, 5.5). All studies report an increase in fat mass and a decrease in LBM in PWS patients compared to normal healthy individuals. This pattern is found in adults, children, and even in PWS infants who are still underweight (Table 2: 2.1, 2.2, 2.3, 2.5, Table 4: 4.1, 4.2, 4.3, 4.5, and Table 5: 5.1, 5.5). In PWS infants, who normally have about 24% body fat, levels of 28–32% are reported (Table 2: 2.1, Table 4: 4.1, 4.2, and Table 5: 5.1). In PWS children, levels of 36–55% are reported, while at this age body fat is normally about 18% (Table 2: 2.4, Table 4: 4.5, and Table 5: 5.5). In both PWS infants and children LBM is 50–60% of total body mass, whereas in normal healthy individuals this is about 80% (Table 2: 2.4, and Table 4: 4.2, 4.5). Moreover, fat distribution is different in PWS patients. Although PWS patients have the same percentage of body fat in comparison to individuals with simple obesity, the fat mass in PWS patients is significantly more increased in the limbs (Table 2: 2.5, 2.8). In contrast, LBM is decreased in all body regions (Table 2: 2.5, 2.8).

The marked increase in fat mass in PWS patients indicates an imbalance between energy intake and energy expenditure. This cannot be solely explained by an increased energy intake since even when PWS patients are underweight in infancy, before hyperphagia develops, body fat is increased (Table 2: 2.1, 2.2). Even more importantly, energy expenditure is decreased in PWS infants, children and adults (Table 2: 2.1, 2.3, 2.4, 2.6, 2.9, and Table 4: 4.5). In normal healthy adults there is a relation between body composition and energy expenditure, LBM is found to be the most predictive variable for Basal Metabolic Rate (BMR).[54] Since BMR is responsible for 70% of total energy expenditure, LBM is the most predictive variable for energy expenditure. With respect to energy expenditure, some authors report that decreased BMR or decreased total energy expenditure can solely be explained by decreased LBM in PWS (Table 2: 2.1, 2.6), whereas others report that LBM is not solely responsible and that PWS patients are probably also less active (Table 2: 2.3, 2.4, 2.9).

Neuromuscular functioning in PWS

Study characteristics

The seven references regarding neuromuscular functioning in PWS belong to four different domains of the motor system: (1) corticospinal excitability and conductivity, (2) muscle tissue, (3) biochemical muscle characteristics, and (4) muscle functioning. A description of the study characteristics is presented in Table 3. Six O-BSD studies were included and one Cohort. PWS patients were compared to norm values, normal control subjects, hypotonic control subjects, and/or obese control subjects. In only two studies more than 20 patients were included.

Methodological quality

Initially the raters reached an agreement of 98% (Cohen's Kappa 0.96) with respect to all the criteria of the seven articles (7 × 6= 42 criteria). 100% consensus was reached following consultation. The quality scores for the articles reached 50–92% of the maximum score.

Methods used

The following methods were used to evaluate neuromuscular functioning in PWS: transcranial magnetic stimulation (TMS) of the motor cortex and motor evoked potential (MEP) to evaluate corticospinal excitability and conductivity;[42] muscle ultrasounds and muscle biopsies to evaluate muscle tissue;[11,24,55] levels of CoQ10, an essential coenzyme for energy synthesis, to evaluate biochemical muscle characteristics;[56] and maximum expiratory and aspiratory pressure and isokinetic dynamometry to assess muscle functioning of thoracic muscles and knee flexors.[57,58]

Results of neuromuscular functioning in PWS

Motor cortex functioning seems to be affected in adult PWS patients. Based on TMS and MEPs the relaxed motor threshold is increased and intracortical facilitation is decreased, which indicates hypo-excitability of the motor cortical areas (Table 3: 3.1). Qualitative studies from muscle biopsies reveal some muscle tissue abnormalities: type-2 fiber atrophy, type-2B fiber deficiency and an increased amount of the immature type-2C fibers (Table 3: 3.3), morphological abnormalities of contractile elements and mitochondria (Table 3: 3.2). Additionally, type-1 fiber size is significantly decreased and variability is increased (Table 3: 3.3). The muscle ultrasound scans were normal, echo intensity was not increased which indicates that muscle tissue is not replaced by fat or fibroses in PWS patients (Table 3: 3.4). These finding were not statistically tested. Compared to normal controls, in muscles of PWS patients CoQ10 levels are decreased, indicating possible mitochondrial dysfunction. The normal association between CoQ10 levels and age was also absent (Table 3: 3.5). Both studies on muscle function report decreased muscle strength: compared to controls muscle force in knee flexors is 70% decreased (Table 3: 3.7), and decreased muscle strength of thoracic muscles in PWS patients leads to impaired pulmonary function (Table 3: 3.6).

Effects of GH treatment on body composition

Study characteristics

The literature search revealed 22 references pertaining to the effects of GH treatment on body composition. In total 23 GH effect studies were evaluated, since one study contained results from an RCT as well as a CS. Of the studies included nine were RCTs (40%) and of the remaining 14 studies the majority were CS. First we present the results of the RCTs, the strongest study design for evaluating treatment effects. The results of the 14 non-randomized GH effect studies are presented subsequently.

Table 3. Characteristics of neuromuscular studies

ID	Study	Design	Sample	Measurements
3.1	Civardi et al., 2004 [42]	O-BSD	**Adults** - 21 PWS (m = 25, 15-39 y) - 11 N-control	Transcranial Magnetic Stimulation (TMS); Motor Evoked Potential (MEP)
3.2	Afifi and Zellweger, 1969 [11]	O-BSD	**Infants** - 7 PWS (m = 5,3) - Norm values	Muscle biopsies (Light microscopy and Electron microscopy)
3.3	Sone, 1994 [24]	Cohort	**Infants** - 11 PWS (m = 13, 8-34 mo) - 8 H-control (m = 13, 8-27 mo)	Muscle type distribution, Morphometric fiber measurement (*muscle biopsies*)
3.4	Heckmatt et al., 1988 [55]	O-BSD	**Infants** - 8 PWS (m = 18 mo) - 214 H-control - Norm values	Muscle ultrasound
3.5	Butler et al., 2003 [56]	O-BSD	**Adults** - 16 PWS (m = 25, 13-44 y) - 13 O-control (m = 27, 13-46 y) - 15 N-control (m = 23, 12-43 y)	H; W; LBM, BF% (DEXA); Coenzyme Q10 levels; Metabolic parameters
3.6	Hakonarson et al., 1995 [57]	O-BSD	**Children-Adults** - 35 PWS (4-54 y) - Norm values	H; W; Pulmonary function; Thoracic muscle functioning
3.7	Capodaglio et al., 2009 [58]	O-BSD	**Adults** - 6 PWS (m = 27, 21-36 y) - 20 O-control (m = 29, 20-40 y) - 14 N-control (m = 30, 23-38 y)	H; W; BMI; muscle strength knee flexor (Isokinetic dynamometer)

Design: O-BSD = Observational Between-Subject Design. **Sample:** m = mean age and age range, N-control = normal control group, O-control = obese control group, H-control = hypotonic control group. **Measurements:** H = height, W = weight, BF% = body fat percentage, LBM = lean body mass, DEXA = dual energy X-ray absorptiometry. **Effect indication:** = = similar, ≠ = different, ↑ = increased, ↓ = decreased, + = positive, - = negative, (X * Y) = correlation between X and Y

Study characteristics of the RCTs

A description of the study characteristics is presented in Table 4. GH was compared to placebo (n=2), or no GH treatment (no-GH, n=5), and two studies examined the dose-dependent effect of GH treatment. The control period varied between 6 (n=3), 12 (n=4) and 24 (n=2) months. In two studies sample size was smaller than 20 patients and in seven studies sample size was larger than 20 patients. Body composition was measured using DEXA, DD, or BIA.

Main findings	Conclusions	Quality
PWS vs. N-control: Relaxed Motor Threshold ↑, Central Motor Conduction Time =, Central Silent Period =, F-wave size =, Intracortical Inhibition =, Intracortical facilitation ↓	TMS changes in PWS patients indicate a hypo-excitability of the motor cortical areas.	75%
PWS vs. norm value: muscle structure with light microscopy =, muscle structure with electron microscopy ≠ (Alternations were found in contractile elements and mitochondria)	The muscle alternations could be compatible with either primary myopathy or neurogenic atrophy, but they could also be secondary to early immobility and disuse.	67%
PWS vs. Norm: Type-1 size ↓, Type-1 size variability ↑, Type-2B deficient, Type-2C ↑; Type-2 atrophy **PWS vs. H-control:** Type-1 size variability ↑, Type-2 atrophy ↑	Muscle fiber immaturity and abnormal muscle fiber type distribution in PWS patients may contribute to muscle hypotonia and weakness.	60%
PWS vs. Norm values: Muscle ultrasound =	Ultrasound scans are normal in patients with PWS.	50%
PWS vs. N-control: CoQ10 levels ↓, LBM ↓ **N-control:** (CoQ10 * Age) + **PWS:** (CoQ10 *Age) ≠ **O-control:** (CoQ10 *Age) ≠ **PWS vs. O-control:** CoQ10 =; LBM ↓	Plasma CoQ10 levels were lower in subjects with PWS.	67%
PWS vs. Norm: Forced Expiratory Volume ↓ (72%), Forced Vital Capacity ↓ (65%), Forced Expiratory Volume/Forced Vital Capacity =, Total Lung Capacity =, Residual volume ↑, Residual volume/ Total Lung Capacity ↑, Thoracic muscle strength ↓	PWS patients have ventilatory impairment primarily as a result of respiratory muscle weakness.	92%
PWS vs. O-control, N-control: Muscle strength knee flexor and extensor ↓, Peak torque ↓, Peak torque/W ↓ (- 70%) **N-control vs. O-control:** Peak torque ↓, Peak torque/W ↑ **In all:** (Peak torque * speed) -, (Peak torque/W * speed) -	Other factors than obesity *per se* seem to contribute to reduced muscular strength in PWS.	83%

Methodological quality RCTs

Initially the raters reached an agreement of 94% (Cohen's Kappa 0.89) with respect to all the criteria of the nine RCTs (9 × 13 = 117 criteria). 100% consensus was reached following consultation. The scores were between 65% and 77% of the maximum score. The selection procedure of patients is not described in any of the RCTs, but from the patient descriptions it is possible to evaluate whether or not the patients included are representative of PWS patients in general. Eight of the studies do not report whether the treatment group and control group were similar with regard to percentage of body fat and LBM before the start of treatment, although the groups were similar with respect to age and gender. None of the RCTs report an intention-to-treat, it is therefore not possible to evaluate whether everyone who received treatment was considered part of the trial, and whether they completed it or not. Since patient loss was absent or small in the RCTs, the absence of an intention-to-treat is not likely to result in misinterpretation.

Table 4. Characteristics of randomized GH effect studies

ID	Study	Sample	Intervention	Measurements
4.1	Whitman et al., 2004 [59]	**Infants** - 18 PWS-GH - 12 PWS-no-GH - Norm values (m = 16, 4-45 mo)	GH vs. no-GH 6 mo	H; W; W/H; BMI; GR; LBM, BF%, BMC (*DEXA*); Motor development; Endocrine/ metabolic parameters
4.2	Carrel et al., 2004 [39] *Suppl. Whitman et al., 2004* [59]	**Infants** - 15 PWS-GH - 14 PWS-no-GH - Norm values (m = 15, 4-37 mo)	GH vs. no-GH 12 mo	H; W; BMI; GR; LBM, BF% (*DD, DEXA, n=14*); TEE (*DLW*); Motor development; Endocrine/metabolic parameters
4.3	Festen et al., 2008 [60]	**Infants** n/a **Children** - 25 PWS-GH - 22 PWS-no-GH - Norm values (m = 6, 4-9 y)	GH vs. no-GH 24 mo	H; W; BMI; head-C; LBM, BF% (*DEXA*); Endocrine/metabolic parameters
4.4	Lindgren et al., 1998 [61]	**Children** - 15 PWS-GH - 12 PWS-no-GH (m = 6, 3-12 y) - 10 O-control (m = 9, 5-12 y)	Observation 6 mo, GH vs. no-GH 12 mo	H; W; GR; BMI; bone age; Endocrine/metabolic parameters **PWS:** Scoliosis; BF%, FFM, BMC (*DEXA, BIA*)
4.5	Carrel et al., 1999 [38]	**Children** - 35 PWS-GH - 19 PWS-no-GH - Norm values (m = 10 y)	GH vs. no-GH 12 mo	H; W; BMI; GR; Bone age; BF%, LBM, BMC (*DEXA*), REE (*IC*); Scoliosis; Strength/agility; Thoracic muscle strength; Endocrine/metabolic parameters.
4.6	Haqq et al., 2003 [62]	**Children** - 6 PWS group A - 6 PWS group B - Norm values (m = 5, 4-15 y)	**A:** GH 6 mo, Placebo 6 mo **B:** Placebo 6 mo, GH 6 mo	H; W; GR; Skinfolds, arm-C; waist-C; hip-C; BF%, LBM, FM, BMC (*DEXA*), REE (*IC*); Pulmonary functioning; Behavior; Cognition; Endocrine/metabolic parameters.
4.7	Carrel et al., 2001 [63] *Suppl. Carrel et al., 1999* [38]	**Children** - 14 PWS-GH-0.3 - 18 PWS-GH-1.0 - 14 PWS-GH-1.5 (m = 11, 5-16 y)	GH 3 y, GH-dosage 12 mo	H; W; BMI; GR; bone age; BF%, LBM, BMC (*DEXA*), REE (*IC, n=26*); Scoliosis; Strength/agility; Thoracic muscle strength; Endocrine/metabolic parameters.
4.8	Carrel et al., 2002 [20] *Suppl. Carrel et al., 2001* [63]	**Children** - 14 PWS-GH-0.3 - 18 PWS-GH-1.0 - 14 PWS-GH-1.5 (m = 11, 5-16 y)	GH 4 y, GH-dosage 24 mo	H; W; BMI; GR; bone age; BF%, LBM, BMC (*DEXA*), REE (*IC, n=26*); Scoliosis; Strength/agility; Thoracic muscle strength; Endocrine/metabolic parameters.
4.9	Höybye et al., 2003 [64]	**Adults** - 9 PWS-GH - 8 PWS-no-GH (17-37 y)	GH vs. Placebo 6 mo, GH 12 mo	W; H; BMI; waist/hip; BF%, LBM (*DEXA*); Endocrine/ metabolic parameters.

Sample: m = mean age and age range, PWS-GH = PWS GH treatment group, PWS-no-GH = PWS control group, O-control = obese control group. **Anthropometric measurements:** H = height, W = weight, GR = growth rate, BMC = bone mineral content, BMI = body mass index, Head-C = head circumference, Arm-C = arm circumference, Waist-C = waist circumference, Hip-C = hip circumference. **Body composition measurements:** FM = fat mass, BF% = body fat percentage, FFM = fat free mass, LBM = lean body mass, LBM-age = lean body mass corrected for age,

Main findings	Conclusions	Quality
PWS vs. Norm: BF% ↑, LBM ↓ **GH vs. n-GH:** T.0: age =, H =, W =, BF% =, LBM =; T.6mo: GR ↑, BF% ↓, Motor dev. ↑ **T.6mo vs. T.0:** GH: H ↑, W ↑, BF% ↓, LBM ↑; no-GH: H =, W ↑, BF% ↑, LBM ↓	GH has a positive effect on growth rate, BF%, and motor development.	65%
PWS vs. Norm: BF% ↑, LBM ↓, TEE ↓ **GH vs. n-GH:** T.12mo: H ↑, BF% ↓, LBM ↑, TEE ↑, Motor dev. = (Subgroup: Motor dev. ↑)	GH improves body composition, and accelerates motor development.	69%
Infants: n/a **Children PWS vs. Norm:** W ↓, H ↓, BMI ↑, Head-C ↓, LBM-age ↓, LBM-height ↓, BF% ↑ **Children GH vs. no-GH:** H↑, LBM-height ↑ **Children T.24mo vs. T0:** GH: H ↑, head-C ↑, LBM-age ↑, LBM-height =, BF% ↓; no-GH: LBM-age ↓, LBM-height ↓	GH-treatment prevents the loss of LBM seen in the non-GH-treated children.	77%
PWS vs. O-control: H ↓ **T0 vs. T.12mo:** GH: H ↑, GR ↑, FFM ↑, BF% ↓, BMI ↓; no-GH: H =, GR =, FFM =, BF% =, BMI =	GH resulted in an increase in growth rate, FFM and a decrease in BMI.	73%
PWS vs. Norm: BF% ↑, LBM ↓, BMD =, REE ↓ **GH vs. n-GH:** GR ↑, BF% ↓, LBM ↑ **T0 vs. T.12mo:** GH: GR ↑, BF% ↓, LBM ↑, BMI =, Bone age =, BMD =, Scoliosis =, REE =, Thoracic muscle strength ↑, Strength/agility ↑	GH caused decrease in body fat and increase in LBM.	69%
PWS vs. Norm: H ↓, Pulmonary function ↓, **GH vs. Placebo:** GR ↑, BMI ↓, BF% ↓, BF ↓, LBM ↑, REE ↑ **T.6mo vs. T0:** GH: Pulmonary parameters ↑	GH improved body composition and REE.	77%
T.3y vs. T0 (12 mo GH-dosage) **GH-0.3:** GR ↓, BF% =, LBM =, Strength/agility = **GH-1.0:** GR ↓, BF% ↓, LBM ↑, Strength/agility = **GH-1.5:** GR ↓, BF% ↓, LBM ↑, Strength/agility = All changes were dose dependent	GH-induced changes in growth and body composition require GH doses of >0.3 mg/m^2/day.	69%
T0 vs. T.4y (24 mo GH-dosage) **GH-0.3:** GR ↓, BF% =, LBM =, Strength/agility = **GH-1.0:** GR ↓, BF% ↓, LBM ↑, Strength/agility = **GH-1.5:** GR ↓, BF% ↓, LBM ↑, Strength/agility = All changes were dose dependent	With GH doses ≥ 1.0 mg/m^2/d, GH-induced changes in body composition can be sustained for 4 y.	69%
GH vs. n-GH: - T.6mo: BF% ↓	GH has beneficial effects on body composition.	77%

LBM-height = lean body mass corrected for height. **Energy measurements:** TEE = total energy expenditure, REE = resting energy expenditure. **Measurement methods:** DD = Deuterium Dilution, DEXA = dual energy X-ray absorptiometry, IC = Indirect Calorimetry, BIA = Bioelectrical Impedance Analysis. **Effect indication:** = = similar, ↑ = increased, ↓ = decreased.

Table 5. Characteristics of non-randomized GH effect studies

ID	Study	Design	Sample	Interv.	Measurements
5.1	Eiholzer et al., 2004 [26]	CCT	**Infants** - 11 PWS-GH (m = 1 y) - 6 PWS-Q10 (m = 0.5 y) - Norm values	GH 30 mo vs. CoQ10 12 mo	H; W; LBM, BF%, BMC (*DD*)
5.2	Myers et al., 2007 [65] *Suppl. Carrel et al., 2004* [39]	CS	**Infants** - 14 PWS-GH (m = 15, 4-37 mo)	GH 24 mo	H; W; W/H; BMI; head-C; LBM, BF%, BMC (*DEXA*); Motor development.; Cognition/language; Endocrine/metabolic parameters
5.3	Eiholzer et al., 1997 [66]	CS	**Children** - 9 PWS-GH (m = 5, 1-7 y)	GH 6 mo	H; W; W/H; GR; Skinfolds; arm-C; BF%, FFM (*DEXA, n=8*)
5.4	Davies et al., 1998 [40]	CS	**Children** - 25 PWS-GH - Norm values (m = 7, 4-10 y)	GH 6 mo	H; W; GR; Skinfolds; BF%, FFM (*DD*)
5.5	Lindgren and Lindberg, 2008 [67] *Suppl. Lindgren*[61]	CS	**Children** - 22 PWS-GH - Norm values (m = 7, 4-13 y)	GH 7 y	H; W; BMI; BF%, LBM, BF/LBM, BMC (*DEXA*); Endocrine/metabolic parameters
5.6	Eiholzer et al., 1998 [41]	CS	**Children** - 3 PWS-under-W-GH (0-4 y) - 6 PWS-over-W-GH (3-7 y) - 3 PWS-GH (9-16 y)	GH 12 mo	H; W; W/H; BMI; BF%, FFM, (*DEXA, n=9*); Endocrine/metabolic parameters; Interview physical activity
5.7	de Lind van Wijngaarden et al., 2009 [68]	CS	**Children** - 55 PWS (m = 6)	GH 4 y	H; W; BMI; head-C; bone age; BF%, LBM, (*DEXA*); Endocrine/metabolic parameters.
5.8	Myers et al., 2000 [69] *Suppl. Carrel et al., 1999* [38]	CS	**Children-Adolescents** - 35 PWS-GH (m = 10, 4-16 y)	GH 24 mo	H; W; BMI; GR, bone age; BF%, LBM, BMC (*DEXA*); REE (*IC, n=16*), Scoliosis, Strength/agility; Thoracic muscle strength (*n=20*) Endocrine/metabolic parameters.
5.9	Eiholzer et al., 2000 [70] *Suppl. Eiholzer et al., 1998* [41]	CS	**Children-Adolescents** - 12 PWS-GH	GH 3.5 y	H; W; W/H; BF%, FM, LBM-height, LBM-age (*DEXA*)
5.10	Galassetti et al., 2007 [71]	Cohort	**Children-Adults** - 21 PWS-GH - 16 PWS-no-GH (3-38 y)	GH 0.6-6.3 y	W; H; BMI; BF%, FM, LBM, BMC (*DEXA*); Food records
5.11	Bosio et al., 1999 [72]	CS	**Children-Adults** - 6 PWS-GH children - 6 PWS-GH adults (6-22 y)	GH 12 mo GH 6 mo	H; W; BMI; BF%, BF%-limbs, BF%-trunk, LBM, BMC (*DEXA*)
5.12	Höybye et al., 2003 [64]	CS	**Adults** - 17 PWS-GH (17-37 y)	GH 12 mo	W; H; BMI; waist/hip; BF%, LBM (*DEXA*); Endocrine/metabolic parameters
5.13	Höybye, 2007 [73] *Suppl. Höybye*[64]	CS	**Adults** - 6 PWS-GH (17-32 y)	GH 5 y	W; H; BMI; waist/hip; BF%, LBM (*DEXA*); Endocrine/metabolic parameters
5.14	Mogul et al., 2008 [74]	CS	**Adults** - 30 PWS-GH (17-49 y)	GH 12 mo	W; H; BMI; waist-C; hip-C; BF%, LBM (*DEXA*); Endocrine/metabolic parameters

Design: CCT = Clinical Controlled Trial, CS = Case Series. **Sample:** m = mean age and age range, PWS-GH = PWS GH treatment group, PWS-Q10 = PWS CoQ10 treatment group, PWS-no-GH = PWS control group. **Anthropometric measurements:** H = height, W = weight, GR = growth rate, BMC = bone mineral content, BMI = body mass index, Head-C = head circumference, Arm-C = arm circumference, Waist-C = waist circumference, Hip-C = hip circumference.

Main findings	Conclusions	Quality
PWS vs. Norm: H ↓, LBM-height ↓, LBM-age ↓, BF% ↑, BF%-age ↑ **T.30 mo vs. T.0:** GH: H ↑, W/H ↑, LBM/H ↑, BF% ↑; Q10: H =, LBM/H ↓, BF%-age ↑	GH therapy in infants lifts LBM corrected for height into the normal range.	79%
Results T.0, T.12mo previously reported **T.24mo vs. T.12mo:** LBM ↑, BF% ↑ **PWS-GH vs. Typical PWS:** First words 14 mo vs. 21-23 mo. Walking 23.5 mo vs. 24-30 mo	The second year of GH treatment in PWS infants caused an increase in LBM; accumulation of excess body fat was reduced but not prevented.	71%
T.6mo vs. T.0: H ↑, GR ↑, W ↓, W/H ↓, Skinfolds ↓, arm-C ↓, BF% ↓, FFM ↑	GH has a positive effect on body composition in patients with PWS.	71%
T.6mo vs. T.0: GR ↑, BF% ↓, FFM ↑	GH treatment resulted in an increase in growth rate, FFM and a decrease in BF%.	71%
PWS vs. Norm: H ↓, BF% ↑, BMI ↑, LBM ↓ **T.12mo vs. T.0:** H ↑, LBM ↑, BMI ↓, BF% ↓, **T.7y vs. T.0 (n = 17):** LBM ↑, BF% =, BF/LBM =	GH treatment normalizes adult height and improves body composition.	79%
T.12mo vs. T.0: Children under-W: H ↑, W/H ↑, Skinfolds ↓; Children-over-W: H ↑, GR ↑, W ↓, W/H ↓, BMI ↓, BF% ↓, FFM ↑, Skinfolds ↓; Adolescents: H ↑, W/H ↑, BF% ↑, FFM ↑, Skinfolds ↓ Parents reported increased physical activity	GH treatment in PWS increases growth rate, height and muscle mass and improves physical performance.	86%
- $BF\%_{SDS}$ decreased the first year and then stabilized - LBM_{SDS} increased the first year, decreased to baseline values the second year and then stabilized - After 4 y height normalized, $BF\%_{SDS}$ >2 SDS, LBM_{SDS} < -2 SDS	GH improves body composition over 4 years by decreasing BF% and stabilizing LBM.	83%
T.12mo vs. T.0: GR ↑, BF% ↓, LBM ↑, REE ↑, strength/agility ↑, Thoracic muscle strength ↑ **T.24mo vs. T.12mo:** GR ↑, BF% =, LBM ↑, REE =, strength/agility ↑, Thoracic muscle strength =	GH treatment increases LBM, decreases BF%, improved strength/agility, and thoracic muscle strength.	79%
- Height normalized after 3.5 y GH - W/H stabilized after 24 mo GH - FM, LBM, stabilized after 12 mo GH - LBM adjusted for height did not increase	GH is not sufficient to normalize lean body mass.	86%
GH vs. no-GH: BMI ↓, FM ↓, BF% ↑, caloric intake ↑ There is no relation between body composition and genetic subtypes.	GH improved body composition, and increased nutrient intake, while these variables were unaffected by genetic subtype.	66%
T.6mo vs. T.0: Children: H ↑, LBM ↑; Adults: LBM-limbs ↑ **T.12mo vs. T.6mo:** Children: H =, LBM ↑	GH might improve final stature and exert a positive influence on body composition.	64%
T.12 mo vs. T.0: BF% ↓, LBM ↑	GH has a positive effect on body composition with no significant side-effects.	79%
T.5y vs. T.0: LBM ↑	Over 5 y GH has sustained favorable effects on body composition.	71%
T.12mo vs. T.0: BF% ↓, LBM ↑	GH improves body composition, and is well tolerated with no glucose impairment.	86%

Body composition measurements: FM = fat mass, BF% = body fat percentage, BF%-Limbs = body fat percentage of limbs, BF%-trunk = body fat percentage of trunk, BF%-age = body fat percentage corrected for age, FFM = fat free mass, LBM = lean body mass, LBM-age = lean body mass corrected for age, LBM-height = lean body mass corrected for height. **Energy measurements:** REE = resting energy expenditure. **Measurement methods:** DD = Deuterium Dilution, DEXA = dual energy X-ray absorptiometry, IC = Indirect Calorimetry. **Effect indication:** = = similar, ↑ = increased, ↓ = decreased.

Results RCTs: effect of GH treatment on body composition

All of the studies included reported beneficial effects of GH treatment on body composition: there is a decrease in the percentage of body fat and an increase in LBM during treatment. For body fat both between-group effects (Table 4: 4.1, 4.5, 4.6, 4.9) and within-group effects are found (Table 4: 4.1, 4.2, 4.3, 4.4, 4.5). In children the percentage of body fat decreases by about 20% during treatment, but remains above normal (Table 4: 4.1, 4.4, 4.5). The effect is far weaker in adults that have not been treated before, about 4% (Table 4: 4.9). Also for LBM between-group effects (Table 4: 4.1, 4.3, 4.6) and within-group effects are reported (Table 4: 4.1, 4.2, 4.3, 4.4, 4.9). In children LBM increases by about 25–40%, but it does not normalize (Table 4: 4.1, 4.4, 4.5). The effect is minimal in adults that have not been treated before, about 3% (Table 4: 4.9). Since GH causes an increase in height, LBM should be corrected for height in infants and children but this was only done in one study. LBM corrected for height did not increase, but since it decreased in the control group a between-group difference was found (Table 4: 4.3). The dose-dependent effect studies of GH treatment demonstrate that a dose of >0.3mg/m2/day is necessary to keep the percentage of body fat below baseline levels and LBM above baseline levels (Table 4: 4.7, 4.8).

Furthermore, small effects of GH treatment on motor performance and energy expenditure have been found. A between-group difference regarding motor development in infants is reported after six months of GH treatment. However after 12 months this effect remains only significant in patients who received GH treatment before 18 months of life (Table 4: 4.1). In children physical strength and agility improved (Table 4: 4.5) and expiratory muscle strength improved (Table 4: 4.5, 4.6). Also in infants and children energy expenditure increased compared to the control group (Table 4: 4.1, 4.2, 4.6).

Study characteristics of the non-RCTs

A description of the study characteristics is presented in Table 5. One CCT, one Cohort, and 12 CS were included. In the CCT GH was compared to CoQ10 supplementation, and in the Cohort GH was compared to no-GH treatment. In the CS body composition in patients was measured at baseline and after a particular time of treatment. The treatment duration differed between studies from 0.5 to 7 years: 0.5 years (n=2), 1 year (n=4), 2 years (n=2) and more than 3 years (n=4). In only two longer study, patients were measured repeatedly during treatment (Table 5: 5.7, 5.9). In nine studies sample size was smaller than 20 patients and in five studies the sample size was larger than 20 patients. Body composition was measured using DEXA or DD.

Methodological quality non-RCTs

Initially the raters reached an agreement of 98% (Cohen's Kappa 0.96) with respect to all the criteria of the 14 non-RCTs ((13 x 7) + (1 x 9) + (1 x 11) = 104). 100% consensus was reached following consultation. The quality scores for the articles reached 64% and 86% of the maximum score. The selection procedure of patients is not described in 12 non-RCTs, but from the patient

descriptions it is possible to evaluate whether or not the patients included are representative of PWS patients in general.

Results non-RCTs: effect of GH treatment on body composition

All authors report positive effects for GH treatment on percentage of body fat and on LBM and therefore support the findings of the RCTs. An effect on body composition compared to baseline is still seen after five years of GH treatment (Table 5: 5.5, 5.13). In two studies the effects of GH were evaluated repeatedly during several years of treatment. It was found that treatment effects on body composition stabilized after the first year (Table 5: 5.7, 5.9). LBM was corrected for height in only two studies with children. Height-corrected LBM increased in infants (Table 5: 5.1) but not in children (Table 5: 5.9). In one study Standard Deviation Scores (SDS) for percentage of body fat and LBM were used, percentage of body fat decreased and LBM remained stable (Table 5: 5.7). Effects of GH treatment on motor performance are also reported: physical strength and agility increased and expiratory muscle strength improved in children (Table 5: 5.8). Moreover, non-significant effects of GH on motor performance were reported. Infants who were treated with GH spoke their first words and walked earlier than is usually seen in PWS (Table 5: 5.2). Parents of children treated with GH reported increased physical activity and performance in their children after starting GH treatment (Table 5: 5.6).

Discussion

Principal findings

Study characteristics

The sample size of the included studies was relatively small, probably due to the low incidence of the syndrome. This is presumably also the reason why the age range was sometimes quite large, and control and follow-up periods were relatively short. The studies on body composition and GH treatment were methodologically well performed and although overlapping outcome variables were used, measurement techniques and age bands differed, and therefore a meta-analysis was not possible. It would be recommendable to determine a standard method for evaluating body composition in PWS patients so that it is possible to perform meta-analysis. The quality of the studies on neuromuscular functioning varied from good to moderate. Since they focused on very different aspects of neuromuscular functioning, there was no overlap in outcome variables.

Body composition in PWS

The first goal of this literature study was to gather insight into the severity of body composition abnormalities in PWS, its relation to age, and its progress over time. Our study revealed nine methodologically well-performed cross-sectional O-BSD reports on body composition in PWS. The reported findings were consistent: PWS patients have increased fat mass and decreased LBM. This pattern is characteristic of the syndrome, since it was found in adults,[18,48,49,52,53] children[17,18,48,49,51-53] and even in PWS infants that are still underweight.[26,39,50,59] Insight into the

severity of the abnormalities in relation to age and its progress over time should ideally be supported by longitudinal studies. We found only cross-sectional reports, which indicate that the relationship between fat mass and LBM becomes more out of balance over time. Two phenomena appear to be responsible for this. First, in normal children body fat percentage is high in infancy and decreases over time in childhood[75,76] in contrast, in PWS children body fat is already increased in infancy, and increases further in childhood. Over time this results in more than double the amount of body fat in children and adults with PWS in comparison to normal individuals. Second, in normal obesity LBM increases proportionally to the increase in fat mass,[77,78] in PWS patients LBM does not increase when fat mass increases. Several studies reported that LBM is only 50-60% of the total body mass in PWS patients instead of 80%, this is 20-30% lower.[19,38,39] Since LBM consists mainly of muscle mass, it can be concluded that muscle mass is decreased by 25-37% in PWS patients ((20/80) x 100% = 25%, (30/80) x 100% = 37%). This presumably affects muscles in all body regions, since LBM is decreased in all body regions,[48,49] and it is assumable that muscle strength is affected. Longitudinal studies on body composition are necessary to confirm these cross-sectional findings.

Neuromuscular functioning in PWS

The second goal of this literature study was to systematically gather studies on neuromuscular functioning in PWS. We only found seven explorative studies focusing on quite distinct subjects. Most studies reported structural and functional muscle abnormalities: morphological abnormalities of contractile elements and mitochondria,[11] type-2 muscle fiber atrophy, with type-2B fiber deficiency and increased immature type-2C muscle fibers[24] decreased type-1 muscle fiber size,[24] decreased CoQ10 levels in muscle tissue indicating abnormal mitochondrial function,[56] and decreased strength of thoracic muscles and knee flexor.[57,58] Only one study reported hypo-excitability of the cortical motor areas.[42] Based on these preliminary results, it can be hypothesized that the clinical symptoms originate at least partly from innate cortical and/or muscle pathology and partly from a secondary phenomenon of disuse. Therefore more research is necessary to study the relationship between the clinical symptoms, hypotonia and decreased muscle mass and strength, and the presence of structural and functional abnormalities in muscles and motor cortex.

Effects of GH treatment on body composition

The third goal of this literature study was to evaluate the reported effects of GH treatment on body composition in PWS patients. Our study revealed 9 methodologically well-performed RCTs and 14 methodologically well-performed non-RCTs. The reported findings were mainly consistent: GH positively influences body composition; it decreases fat mass and increases LBM. Nevertheless some critical remarks must be made. First, in children the effect of GH on body fat is much larger (20%),[38,59,61] than in adults (4%),[64] however, even in children, body fat percentage does not normalize. Second, although for all studies the effect of GH on LBM is reported to be

much larger in children than in adults, the actual effect seems to be overestimated because LBM is usually not corrected for height, despite GH causing an increase in growth rate. In three out of four studies in which LBM was corrected no increase was found.[60,68,70] Third, after the first treatment year there is no additional effect of GH on body composition,[70] but the initial effect on body fat and LBM remains stable over time.[67,73] Therefore, GH seems to prevent LBM and body composition becoming more out of balance over time, a phenomenon normally seen in PWS. Furthermore, GH deficiency in PWS is probably not the only cause of body composition abnormalities, since GH treatment completely normalizes length but does not normalize body composition.[67] More research is necessary to further evaluate the long-term effects of GH treatment on body composition and the presumed effect on muscle mass.

This article in relation to the literature

This is the first systematic review on body composition and neuromuscular functioning in PWS. In fact, to our knowledge, there is only one other systematic review on autism in PWS.[79] Comparison can be made to some non-systematic reviews on body composition and GH treatment in PWS.[25,80,81] In general their findings are in line with ours but they do not provide insights into the severity of body composition abnormalities, its relation to age, its progress over time, and the implications of correcting LBM for height. We found one opinion based review in which it is proposed, that decreased muscle strength could be the result of abnormal protein synthesis in muscle tissue as result of decreased GH secretion.[23] In our systematic literature no evidence was found for this suggestion.

Understanding motor problems in PWS

We performed this review to answer the question to which extent abnormal body composition and abnormal neuromuscular functioning may contribute to the motor problems in PWS. We found evidence that muscle mass is decreased by about 25-37%. It is suggested that this decrease is related to GH deficiency in PWS.[25-27] However, also structural and functional abnormalities in muscles are found.[11,24,56-58] Although there is a rough linear relationship between muscle strength and muscle cross-sectional area,[82] in our opinion, decreased muscle mass cannot be the only reason for decreased muscle strength in PWS patients. For example, Capodaglio et al.[58] reported that muscle strength in the knee flexor was 70% lower than normal and therefore much more affected than expected based on decreased muscle mass alone. Inactivity may also contribute to both decreased muscle mass and muscle strength, since PWS infants are found to be less active even before birth.[83] This inactivity may also negatively influence motor performance and development of cortical motor areas and the neuromuscular systems.[84-87] Another aspect that could contribute to motor problems in PWS is the fact that although PWS patients need more muscle strength in daily activities, because of their overtime increasing BMI, the ratio between LBM and fat mass decreases overtime. In the literature there is a strong focus on body composition abnormalities as result of GH deficiency in PWS as cause of the motor problems.

Indeed they do contribute to it, but cannot totally explain them. Research in PWS should therefore focus on other possible causal factors. Another interesting finding in this respect is that in PWS motor performance is found to improve over time although body composition becomes more out of balance over time. Motor problems are most striking in infancy, in which motor development is dramatically constraint by severe hypotonia and muscle weakness, with as result that infants are almost motionless, because they cannot overcome gravity.[10,11,13] Especially in this period of life motor activity is a strong stimulator for cortical and muscular development, the so-called sensitive period.[84-87] In our opinion it is very likely that motor problems in PWS are also related to innate central nervous system defects, in the same manner as endocrine abnormalities are related to defects of the hypothalamus.[25-27] Indeed, there are some structural brain abnormalities found in PWS patients,[28-32,88,89] but only one study reports a possible relationship between brain abnormalities and hypotonia.[89] The hypo-excitability of cortical motor areas in PWS patients are hypothesized as of neurogenetic origin,[42] although these findings can also be related to disuse and problematic development.

Due to the strong dominant focus on body composition as contributing factor, and the beneficial results from GH on body composition, it is thought that GH treatment could also positively affect muscle strength and motor performance.[38,39,41] Although beneficial effects of GH treatment on motor performance have been reported,[38,39,59,69] it is questionable whether there is a direct relationship between GH treatment, muscle mass, muscle strength and motor performance. We are of the opinion that patients will benefit more from GH treatment when also participating in a training program, since it has been shown that also training has a positive effect on body composition in PWS patients[90-92] and training prevents disuse. Since GH treatment alone does not normalize body composition it is possible that GH treatment and training enhance one another and the combination of the two will lead to better results for both body composition and motor performance. Moreover, we advocate that especially young infants can profit from early intervention focusing on skill acquisition and strength training to increase motor development, because in the sensitive period the effect of training influences cortical and muscular development most.[84-87] To get more insight in the relationship between the clinical signs and symptoms and the structural and functional impairments, training and the effect of training on muscle strength, muscle tissue and cortical motor areas should be monitored not only with clinical relevant outcome measurements, but also with measurements focusing on structural and functional changes in muscles and cortex using non-invasive muscle ultrasound scans[93,94] and functional MRI.

General conclusions

In conclusion, the research on body composition and the effect of GH has brought a lot of benefits for patients with PWS in managing the imbalance between fat mass and LBM, which in general has a positive effect on motor performance and fitness. However, insight in the origin of severe motor problems and hypotonia in infancy, and decreased muscle mass and strength in

children, adolescents, and adults with PWS is poor at this moment. We found some preliminary findings that decreased muscle mass, hypotonia, muscle weakness and motor problems in PWS are related to impairments in the nervous and muscular system. Especially the combination of mental retardation and psychiatric disorders, both characteristic for PWS, point into the direction of brain pathology. Research focused on functional and structural abnormalities of cortical motor areas in PWS patients is therefore needed. It seems also that the abnormalities in muscle mass and strength are related to structural and/or functional abnormalities in muscles. However, the interaction between (dis)use and the more structural and functional abnormalities seems to be a clinical relevant finding, since it has been shown that training also has a positive effect. Future research should be focused on getting insight in the relationship between clinical signs and symptoms, and the structural and functional abnormalities in the motor system.

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Appendix A

Search strings

Pubmed	((((Muscle[tiab] OR Muscles[tiab]) OR (Musculoskeletal[tiab]) OR ("Body Composition"[tiab])) AND ((Prader Willi Syndrome[mesh]) OR (("Prader"[tiab] AND "Willi"[tiab]) AND ("Syndrome"[tiab])))) OR (((Musculoskeletal and Neural Physiological Phenomena[mesh]) OR (Musculoskeletal System[mesh]) OR (Musculoskeletal Development[mesh]) OR (Musculoskeletal Physiological Phenomena[mesh]) OR (Body Composition[mesh])) AND ((Prader Willi Syndrome[mesh]) OR (("Prader"[tiab] AND "Willi"[tiab]) AND ("Syndrome"[tiab])))) Limit: English, Publication Date from 1956/1/1 to 2009/12/31
Chinahl	(AB Muscle or AB Musculoskeletal or AB "Body Composition") and (TI Prader and TI Willi and TI Syndrome) Limit - Published Date from: 195601-201001; Language: English Search modes - Boolean/Phrase
Psycinfo	(Muscle or Muscles or Body Composition or Musculoskeletal).ab. and (Prader and Willi and Syndrome).ti. Limit 24 to (english language and yr="1956 – 2010")
Embase	(Muscle or Muscles or Body Composition or Musculoskeletal).ab. and (Prader and Willi and Syndrome).ti. Limit 24 to (english language and yr="1956 – 2010")

3

The effect of growth hormone treatment or physical training on motor performance in Prader-Willi syndrome: A systematic review

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Abstract

Although motor problems in Prader–Willi syndrome (PWS) are prominent in infants, and continue into childhood and adulthood, there is little insight into the factors important for clinical management. The literature was reviewed to: (1) provide an overview of the characteristics and prevalence of motor problems and (2) evaluate the effects of growth hormone (GH) treatment and physical training on motor performance. A systematic search revealed 34 papers: 13 on motor performance; 12 on GH treatment; and nine on physical training. In infants, motor development is 30–57% of the normal reference values, and children and adults also have significant problems in skill acquisition, muscle force, cardiovascular fitness, and activity level. GH treatment positively influenced motor performance in infants, children, and adults, although not all studies demonstrated an effect. All studies on physical training demonstrated beneficial effects in PWS patients. We suggest a combination of GH treatment and physical training to be started as soon as possible, especially in infants, to improve motor development as this will positively influence general development.

Introduction

Prader–Willi syndrome (PWS) is a neurogenetic disorder characterized by decreased motor performance due to hypotonia and muscle weakness. These symptoms are especially striking in infants and continue into childhood and adulthood. Because of low activity levels in PWS patients and later on in life increased prevalence of severe obesity, insight into the influence of motor performance problems in daily life, and the possible effective treatment options, is highly important.

PWS results from an absent expression of the paternal region of chromosome 15q11–13 and is characterized by a wide variety of physical, cognitive, and behavioral defects. The most significant characteristics are hypotonia, hypogonadism, obesity, short stature, cognitive deficits, and mild dysmorphic facial features.[1-3] The estimated prevalence is 1:10,000–30,000 births.[4] Before the introduction of genetic tests, PWS was diagnosed based on clinical features alone using strict clinical determined criteria.[3] In a large retrospective study with genetically confirmed PWS patients, the clinical features of PWS were reported to be highly sensitive.[5] The amount of patients with a clinically positive diagnosis of PWS, in which the genetic test will be negative, is thought to be very small.[5] Therefore, there is a small chance that in older studies, before the introduction of genetic tests, some patients without PWS have been included. We do not think this will influence the conclusions. The severity of disabilities differs between patients and the presentation of the impairments is mostly age-dependent. In infancy, motor problems are most prominent. Patients suffer from severe muscular hypotonia, muscle weakness, poor sucking reflexes, and feeding difficulties. As a result, infants fail to thrive and their psychomotor development is seriously delayed.[6-10] In childhood, most patients develop hyperphagia, which without diet restrictions, can lead to severe obesity in combination with severe inactivity.[1,3,11] In addition, cognitive and social–emotional deficits, as well as behavioral problems are present.[1,11,12] In adulthood, there is a lack of complete pubertal development.[13,14] Cognitive emotional, or even psychiatric problems are common.[1,15,16] To improve the health and quality of life of PWS patients, the management of this complex disorder requires an age-dependent, multidisciplinary approach.[1,17] Therefore, the perspectives of different health professionals on the relationships between the diverse symptoms in PWS need to be structured and integrated in clinical management. The International Classification of Function, Health, and Disability (ICF) of the World Health Organization is a useful tool to describe the complex interaction between the individual health condition and contextual factors, as well as personal factors.[18,19] The ICF provides three levels of evaluation: body functions and structures, activity, and participation, and can also be used for clinical reasoning and research in PWS. Although we are aware of the strong interaction between the different developmental domains (physical, cognitive, emotional, and social) in this review, we focus on motor performance in PWS in particular. For an overview of intellectual functioning, neurobehavioral profile, behavioral disturbances, and psychiatric disorders related to the genetic profile, we recommend the review of the literature in a recent article of Cataletto et al.[2]

Although motor performance is normally described at the ICF level of age-related activities, such as the capacity to crawl, walk, cycle, swim, or perform work- and sport-related skills,[18,19] in PWS studies, research is merely focused on the ICF level body functions and structures, e.g. body composition, muscle weakness, and hypotonia.[1,3,17] Motor developmental patterns in PWS infants are rarely studied and the impact of impaired motor development on the activity and participation level of PWS children is unclear. Since motor problems are very prominent in infancy, and hypotonia and decreased muscle mass are still present in childhood and adulthood,[20-22] we hypothesize that motor performance remains affected when these children are growing up.[23] Although clearly recognized in clinical management in childhood and adulthood, motor performance problems are only marginally described in the literature. Some studies report that PWS patients are significantly less active compared with normal individuals,[24-28] and score significantly lower on standardized motor performance tests.[29,30]

It seems logical that decreased motor performance in PWS patients is related to their abnormal body composition.[17,23] From birth onwards, fat mass is increased[31-34] and muscle mass is decreased by about 25–37%.[23] Abnormal body composition in PWS is thought to be related to growth hormone (GH) deficiency.[35-37] GH treatment in PWS patients has a positive influence on body composition, but does not normalize it.[23,34,38,39] It is presumed that GH treatment could also have a positive effect on muscle strength, and thereby on motor development and/or performance.[32,38,40] On the other hand, it is also possible that the severe inactivity contributes to a lower performance and fitness level, since PWS patients are found to be less active,[24-28] even before birth.[41] A vicious circle seems to exist: the abnormalities in muscle force and body composition lead to motor performance problems and these problems hinder the PWS children from participating in leisure activities and sports. This leads to even more loss of muscle force, lower physical fitness, decreased skill acquisition, inactivity, and adiposity. Therefore, it can be hypothesized that physical training and pleasure in active exercises would be beneficial.

It is well known that in healthy individuals, body composition, muscle force, skill acquisition, and fitness level can be influenced by physical training.[42,43] There are some indications that this is also possible in PWS patients.[44-46] It can be expected that physical training potentially has a positive influence on both body structures and function and thereby on the activity and participation level. Unfortunately, there are hardly any studies focusing on intervention programs to improve motor development or motor performance in PWS.

To our knowledge, the effects of GH treatment or physical training in PWS patients concerning motor performance, muscle strength, and physical fitness, have never been studied systematically. Therefore, we decided to perform a systematic review of the literature.

The aims of this literature study were to provide: (1) an overview of the reported findings concerning motor performance in PWS; (2) an evaluation of the effects of GH treatment on motor performance in PWS patients; (3) an overview of physical training programs evaluated in PWS patients and their results; and (4) recommendations for intervention and research programs to improve motor performance, activity, and participation in PWS patients.

Methods

Search strategy

Relevant papers were identified through two separate literature searches: (1) motor performance in PWS and (2) physical training in PWS. The first not only revealed studies focusing solely on motor performance in PWS, but also articles concerning the effects of GH treatment on motor performance in PWS. For both, we conducted a search of English language publications covering the period from January 1980 to July 2011 using four electronic databases: PubMed, Embase, CINAHL, and PsycINFO. Since only Embase and PsycINFO used the same thesaurus terms and major subheadings, searches were performed separately in each database and results were combined afterwards. The search strings are listed in Appendix A. In addition, the reference lists of relevant papers were manually checked for further articles not previously identified.

Selection criteria

In both searches, overlapping articles were deleted. Each abstract was screened by two authors (L.R. and M.N.) and articles were included when they concerned an original scientific study on motor performance in PWS, or the effect of GH treatment or physical training in PWS. Only articles reporting original data were included. A publication was excluded if it concerned a case report of only one patient, narrative reviews, or animal research, or when there was no abstract available. Furthermore, articles were excluded when the focus was not specifically on PWS, or when the main object was out of the scope of this study: for example, when a study focused on the genetic aspects of PWS, behavior, cognition, language, hyperphagia, diet, obesity, or hormonal functioning. After selection, the full text article was screened again, based on the inclusion and exclusion criteria.

Evaluation of the methodological quality

The methodological quality of the included references was evaluated based on the technical aspects of the study design. Overall, we included five different study designs: randomized controlled trials (RCT); controlled clinical trial (CCT); cohort study (CoS); case series (CS); and observational between-subject design (O-BSD). Standard checklists were not available for all of these designs, so we constructed a new checklist with criteria from three standard checklists: the Dutch Evidence-Based Guideline Development checklist (EBRO: randomized controlled trial, case–control study, Cohort study),[47] the Physiotherapy Evidence Database Scale (PEDro),[48] and the Critical Appraisal Skills Programme (CASP)[49]. The criteria used per design and the assignment of ratings are presented in Table 1. We used 13 criteria to evaluate the methodological quality of the studies: (1) randomization; (2) group definition; (3) selection bias; (4) group comparability; (5) treatment procedure; (6) blinding; (7) control period; (8) study duration; (9) measurement procedure; (10) comparability of treatment; (11) confounding effects; (12) loss of patients; and (13) intention-to-treat analysis.

Table 1. Evaluation of the methodological quality

Study	Design	1 Random-ization	2 Group definition	3 Selection procedure	4 Group comparability	5 Treatment procedure	6 Blinding
Motor performance in PWS							
Festen et al., 2007 [54]	O-BSD	-	2	1	2	-	-
Chen et al., 2010 [55]	O-BSD	-	2	1	2	-	-
Ehara et al., 1993 [50]	O-BSD	-	2	1	1	-	-
Van Mil et al., 2000 [28]	O-BSD	-	2	1	2	-	-
Davies & Joughin, 1993 [25]	O-BSD	-	2	1	2	-	-
Cimolin et al., 2011 [58]	O-BSD	-	2	1	2	-	-
Capodaglio et al., 2011 [59]	O-BSD	-	2	1	2	-	-
Cimolin et al., 2010 [61]	O-BSD	-	2	1	2	-	-
Capodaglio et al., 2009 [20]	O-BSD	-	2	1	1	-	-
Butler et al., 2007 [24]	O-BSD	-	2	1	2	-	-
Vismara et al., 2007 [63]	O-BSD	-	2	1	2	-	-
Hakonarson et al., 1995 [64]	O-BSD	-	2	1	2	-	-
Greenswag, 1987 [51]	CoS	-	2	2	1	n/a	n/a
Effects of GH treatment on motor performance in PWS							
Festen et al., 2008 [10]	RCT	2	2	1	2	2	0
Myers et al., 2007 [53]	RCT	2	2	1	2	2	2
Carrel et al., 2004 [32]	RCT	2	2	1	2	2	2
Whitman et al., 2004 [34]	RCT	2	2	1	2	2	0
Carrel et al., 2002 [29]	RCT	2	2	1	0	2	2
Carrel et al., 1999 [38]	RCT	2	2	1	2	2	2
Carrel et al., 2010 [52]	CoS	-	2	1	1	2	2
Eiholzer et al., 2008 [9]	CoS	-	2	1	2	2	1
Gondoni et al., 2008 [65]	CS	-	2	1	-	2	-
Eiholzer et al., 2001 [8]	CS	-	1	1	-	2	-
Myers et al., 2000 [56]	CS	-	2	1	-	2	-
Eiholzer et al., 1998	CS	-	2	1	-	2	-
Physical training in PWS							
Grolla et al., 2011 [66]	CS	-	2	1	-	2	-
Capodaglio al., 2011 [60]	CCT	-	2	1	1	2	0
Vismara et al., 2010 [62]	CCT	-	2	1	2	2	0
Schlumpf et al., 2006 [45]	CCT	-	2	1	2	2	0
Eiholzer et al., 2003 [44]	CCT	-	2	1	2	2	n/a
Silverthorn & Hornak, 1993 [46]	CCT	-	2	1	0	2	0
Kaufman et al., 1995 [67]	CS	-	1	1	-	1	-
Mullins and Vogl-Maier, 1987 [30]	CS	-	1	0	-	2	-
Caldwell et al., 1986 [68]	CS	-	2	1	-	2	-
Nardella et al., 1983 [57]	CS	-	2	1	-	2	-

The methodological evaluations of all included articles categorized per topic: 1) motor performance, 2) GH treatment, and 3) Physical training. Evaluation: - = the criterion was not relevant for this design type, n/a = the criterion was not relevant in this particular study, 0 = zero points were assigned since the criterion was not described, 1 = one point was assigned since the criterion was not completely described and/or the fulfillment was doubtful, 2 = two points were assigned since the criterion was well described and fulfilled, % = the percentage of the maximum score. For example: the maximum score for a RCT is 26 points (13 x 2 points). If a RCT had a score of 18 points, it was evaluated as 69 percent.

7 Control or training period	8 Study duration	9 Measurement procedure	10 Comparability of treatment	11 Confounding effects	12 Patient/ data loss	13 Intention-to-treat-analysis	Quality
-	-	2	-	1	1	-	75%
-	-	2	-	1	1	-	75%
-	-	1	-	0	1	-	50%
-	-	2	-	2	1	-	83%
-	-	2	-	2	1	-	83%
-	-	2	-	2	1	-	83%
-	-	2	-	2	1	-	83%
-	-	2	-	2	1	-	83%
-	-	2	-	2	2	-	83%
-	-	2	-	1	1	-	75%
-	-	2	-	2	1	-	83%
-	-	2	-	2	2	-	92%
-	n/a	1	n/a	0	2	-	75%
2	2	2	2	2	2	0	81%
2	2	2	2	1	1	0	81%
2	2	2	2	1	1	0	81%
2	2	2	2	0	1	1	73%
2	2	2	2	1	1	0	73%
2	2	2	2	1	1	0	73%
-	2	2	2	2	1	-	85%
-	2	2	2	2	1	-	85%
-	2	2	-	1	2	-	86%
-	2	1	-	0	1	-	57%
-	2	2	-	2	1	-	86%
-	2	2	-	0	1	-	71%
-	2	2	-	1	1	-	79%
2	2	2	2	1	1	1	71%
2	2	2	2	1	1	1	71%
2	2	2	2	2	1	1	79%
2	2	2	2	2	1	n/a	82%
2	2	2	2	2	1	1	71%
-	2	1	-	2	1	-	64%
-	2	2	-	0	1	-	57%
-	2	1	-	1	1	-	71%
-	2	2	-	0	2	-	76%

For study evaluation, all 13 criteria were relevant and used for an RCT; 12 criteria were used for a CCT; 10 criteria were used for a CoS; seven criteria were used for a CS, and six criteria were used for an O-BSD.

Two authors (L.R. and L.V.) independently scored each article using the checklist. First, it was established whether a criterion was reported. Two points were assigned when the criterion was well described and fulfilled. One point was assigned when the criterion was not completely described and/or the fulfillment was doubtful. Zero points were assigned when the criterion was

not described. The level of agreement between the two raters was determined by Cohen's kappa coefficient. To compare the evaluations of different study designs, the percentage of the maximum score was calculated. For example: the maximum score for an RCT is 26 points (13 × 2 points). If an RCT had a score of 18 points, it was evaluated as 69%.

Presentation of the results

The data are presented in the following order. First, a summary of the results of our electronic database search. Second, an evaluation of the methodological quality of the included studies. Then the findings on motor performance in PWS, effects of GH treatment, and effects of physical training are reported.

For each topic, we present the included articles in reference tables (Tables 3–5), and describe their general characteristics. The summary of the findings on the motor performance studies is categorized per age group (infants, children, adolescents, and adults) and motor performance domain (skill acquisition, muscle strength, activity level, and physical fitness). For the GH treatment effect studies, the findings are summarized in the same manner, but first the findings of the strongest design types are evaluated. Concerning physical training in PWS, we summarized the content of the physical training programs and their results.

Results

Results of electronic databases search

The literature search on motor performance in PWS identified 115 unique references of which 25 met our inclusion criteria. The literature search on physical training in PWS revealed 24 references of which 10 met our inclusion criteria, so in total 35 studies were included. The selection procedure is presented in Figure 1. Table 2 presents the exclusion criteria and the number of excluded articles.

To provide an overview of motor performance in PWS, 24 articles were used: 13 studies solely focusing on motor performance and the baseline measurements of six GH treatment effect studies, and five physical training studies. Because in these intervention studies, motor performance of PWS patients was compared with normal healthy individuals. To evaluate the effects of GH treatment, 12 GH treatment effect studies were included. The nine physical training studies were included to present an overview of the physical training programs and their results.

Methodological quality

The evaluation of the methodological quality of all 35 included studies is presented in Table 1. Only one study described the selection procedure of patients. For the other studies, one point was assigned for the selection procedure when we considered the included patients as being representative of PWS patients. This means that in all studies PWS was clinically confirmed and in the more recent studies also genetically. In six studies, it was discussed whether and why there was any patient loss.

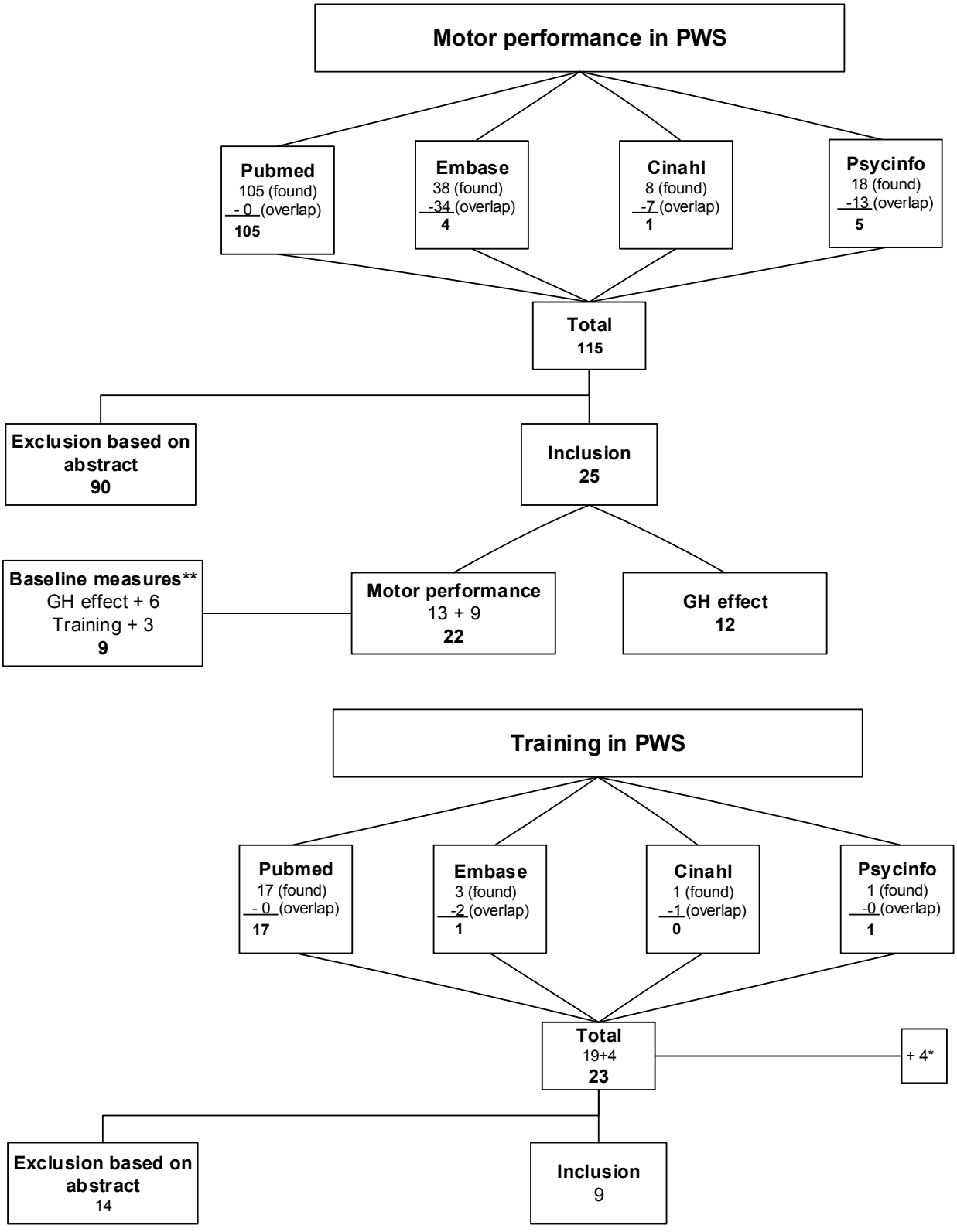

Figure 1. Flow chart selection procedure

* Articles included from reference lists.

** In these GH treatment and physical training studies, motor performance of PWS patients was compared with normal individuals at baseline.

Table 2. Excluded articles and reasons for exclusion

Reasons for exclusion	Number of articles excluded
Motor performance in PWS	
Case report	7
Research in animals	6
No abstract available	2
Different focus:	
Syndrome different from PWS/not PWS specific	22
Genotype	16
Social behavior/cognition/language	22
Hyperphagia/diet/obesity	3
Hormonal functioning	3
Other	9
Total	**90**
Physical training in PWS	
Case report	3
Different focus:	
Social behavior/cognition/language	1
Hyperphagia/diet/obesity	4
Other	6
Total:	**14**

Since the result tables of the other studies showed no patient loss, we concluded that in these studies, all patients were present at the end and therefore one point was assigned.

For the 13 studies solely focusing on motor performance in PWS patients, the raters initially reached an agreement of 90% (Cohen's kappa coefficient 0.79) with respect to all criteria of the eight O-BSDs and one retrospective CoS (13 × 6 = 78 criteria). Complete (100%) consensus was reached following consultation. The methodological quality scores varied between 50% and 92% of the maximum score and three possible risks were found. First, since the patient selection procedure was only described in one study, there is risk of selection bias. Second, it is possible that patient loss influenced the results of the studies. Third, in three studies, it was not possible to evaluate whether the groups were comparable since this was not statistically tested.[20,50,51]

For the 12 studies focusing on the effectiveness of GH treatment, the raters initially reached an agreement of 90% (Cohen's kappa coefficient 0.80) with respect to all criteria of the six RCTs, two CoSs and four CS ([6 × 13] + [2 × 10] + [4 × 7] = 126 criteria), and 100% consensus was reached following consultation. The methodological quality scores varied between 57% and 86% of the maximum score and four possible risks were found. First, there is a risk of selection bias, because none of the studies described the patient selection procedure. Second, only two studies reported patient loss, which possibly influences the results. Third, in four studies, it was not possible to evaluate whether the groups were comparable with respect to motor performance before the start of treatment.[32,34,52,53] Fourth, in five of the RCTs an intention-to-treat analysis was not reported, which could lead to misinterpretation of the results.

For the 10 studies focusing on physical training in PWS patients the raters initially reached an agreement of 88% (Cohen's kappa coefficient 0.77) with respect to all criteria of the five CCTs, and five CS ([5 × 12] + [5 × 7] = 95 criteria); and 100% consensus was reached following consultation. The quality scores varied between 57% and 82% of the maximum score and two possible risks were found. First, there is a risk of selection bias, since none of the studies described the patient selection procedure. Second, it is possible that patient loss influenced the results of the studies, since this was only reported in one study.

Motor performance in PWS

Study characteristics

A description of the study characteristics is presented in Table 3. The 13 studies solely focused on motor performance: 12 O-BSDs and one retrospective CoS. Taken together, with the baseline reports in the intervention studies, six studies focused on infants, seven on children/adolescents, and seven on adults. The findings in these studies can be arranged into four different motor domains: skill acquisition (n=12), muscle strength (n=2), activity level (n=7), physical fitness (n=1), both activity level and physical fitness (n=1), and both skill acquisition and muscle force (n=1). In the majority of these studies, motor performance of PWS patients was compared with healthy peers using normal reference values or age-matched controls. PWS patients were compared with obese individuals in two studies, both healthy and obese controls in three studies, and both healthy controls and patients with Down syndrome in two studies. In 70% of the studies, sample size was relatively small: fewer than 20 PWS patients were included.

Results: motor performance in PWS

SKILL ACQUISITION. Of the 11 studies concerning skill acquisition, six focused on infants and five on adults. Skill acquisition in infants was mostly evaluated using standardized motor develop-mental tests. Scores on these tests were very low: 30–57% of the normal reference values (Table 3: 1–3, 5, 6). Only one study used a non-standardized parents' questionnaire concerning the achievement of motor milestones. In this questionnaire, parents reported seriously delayed motor milestones in their infants: head control at 8 months; rolling at 11 months; crawling at 19 months; walking at 32 months (Table 3: 7).

Skill acquisition is also affected in adults with PWS. In a survey among parents of 232 adult PWS patients, it was reported that PWS adults have poor gross motor skills. However, which gross motor skills were affected was not described (Table 3: 24). It was also retrospectively reported that in infancy patients were floppy and motor development was delayed: they were able to sit at 12 months and walk at 27 months (Table 3: 24). Furthermore, balance capacity was reduced in PWS adults compared with both normal and obese controls. The data were adjusted for individual height and foot length because PWS patients are short and have small feet, which both influence balance capacity.

Table 3. Motor performance

ID	Study	Design	Sample
1	Festen et al. (2008) [10]	Baseline results RCT	**Infants** - 16 PWS-GH - 18 PWS-n-GH - Normal values (m = 2, 1-3 years)
2	Eiholzer et al. (2008) [9]	Baseline results CoS	**Infants** - 6 PWS-GH, Weight < 0 SDS (m = 0.7 years) - 12 PWS-GH, Weight = 0 SDS (m = 1.3 years) - 8 PWS-CoQ10, Weight = 0 SDS (m = 0.6 years) - Normal values
3	Festen et al. (2007) [54]	O-BSD	**Infants** - 4 PWS-OSA - 18 PWS-n-OSA - Normal values (m = 1.8, 1-4 years)
4	Carrel et al. (2004) [32]	Baseline results RCT	**Infants** - 15 PWS-GH - 14 PWS-n-GH - Normal values (m = 15, 4-37 months)
5	Eiholzer et al. (2001) [8]	Baseline results CS	**Infants** - 10 PWS-GH - Normal values (m = 1, 0-4 years)
6	Chen et al. (2010) [55]	O-BSD	**Infants/Children** - 10 PWS (m = 4.8, 1-6 years) - 11 N-control (m = 3.4 years) - Normal values
7	Ehara et al. (1993) [50]	O-BSD	**Infants/Children** - 11 PWS - Normal values (1-13 years)
8	Carrel et al. (1999) [38]	Baseline results RCT	**Children** - 35 PWS-GH - 19 PWS-n-GH - Normal values (m = 10, 4-16 years)
9	Mullins and Vogl-Maier (1987) [30]	Baseline results CS	**Children:** - 8 PWS - Normal values (8-13 years)
10	Eiholzer et al. (2003)	Baseline results CCT	**Children/Adolescents** - 17 PWS (m = 10, 4-19 years) - 18 N-control (m = 11, 5-20 years)
11	Myers et al. (2000) [56] *Suppl. Carrel et al. (1999)*	Baseline results CS	**Children/Adolescents** - 35 PWS-GH - Normal values (m = 10, 4-16 years)
12	Van Mil et al. (2000) [28]	O-BSD	**Children/Adolescents** - 17 PWS - 17 O-control (m = 12, 7-20 years)
13	Davies and Joughin (1993) [25]	O-BSD	**Children/Adolescents** - 10 PWS (m = 12, 6-16 years) - 60 N-control (m = 13, 7-18 years)

Motor measurements	Main findings	Conclusions
Psychomotor dev. (BSID II)	**PWS vs. Norm:** Mental dev. ↓72%, Motor dev. ↓ 57%	Motor development is seriously delayed in PWS infants.
Psychomotor dev. (Griffith test)	**PWS vs. Norm:** Global dev. ↓ 54-66%, Motor dev. ↓ 30-44%, Social dev. ↓ 60-80%, Speech dev.↓ 55%, Hand-eye coordination ↓ 68%, IQ ↓ 57-70%	Psychomotor development is seriously delayed in PWS infants.
Psychomotor dev. (BSID II), Polysomnography	**PWS vs. Norm:** Motor dev. ↓ 55%, Mental dev. ↓ 73% **PWS:** In all patients sleep-related breathing disorders, (Breathing disorder × Psychomotor dev.) ≠	Psychomotor development in PWS infants is delayed and not related to breathing disorders.
TEE (DLW)	**PWS vs. Norm:** TEE ↓	PWS infants demonstrate reduced TEE compared with predicted values for age- and weight-matched normal values.
Psychomotor dev. (Griffith test)	**PWS vs. Norm:** Global dev. ↓ 60%, Motor dev. ↓ 40%, Social dev. ↓ 60%, Speech dev.↓ 50%, Hand–eye coordination ↓ 65%	Especially motor and speech development are seriously delayed in PWS infants.
Metal dev.; Motor dev. (BSID II, WPPSI-R, CCDI)	**PWS vs. Norm:** Global dev. ↓ 60%, Mental dev. ↓72%, Motor dev. ↓ 57%, Gross motor dev. 53%, Fine motor dev. 68%	PWS patients show an uneven global developmental delay.
Motor milestones (Questionnaire)	**PWS vs. Norm:** Motor dev. ↓ Head control 8 months, Rolling 11 months, Crawling 19 months, Walking 32 months	Developmental motor milestones are delayed in PWS infants.
REE (IC)	**PWS vs. norm:** REE ↓	In PWS children, REE is about 60% of predicted kilocalorie utilization corrected for body surface area.
Cardiovascular fitness (Shuttle run test)	**PWS vs. norm:** Shuttle run test ↓	PWS children perform below the first percentile on the elementary school norms for the shuttle run test.
Walking distance (Pedometer); Activity record; Exercises repetitions	**PWS vs. N-Control:** Walking distance ↓ 50%, Activity record ↓, Exercises repetitions ↓ 31%	In PWS children, daily walking distance and exercises capacity is seriously decreased compared with normal healthy children.
REE (IC, n=16)	**PWS vs. Norm:** REE ↓	In PWS children, REE is about 51% of predicted kilocalorie utilization corrected for body surface area.
BMR (IC); ADMR (DLW); PAL [ADMR/BMR]; AEE [0.9 ADMR – BMR]	**PWS vs. O-control:** BMR ↓, ADMR ↓, PAL ↓, AEE ↓	EE is decreased in PWS patients, because of decreased fat free mass and probably also because of reduced physical activity.
TEE (DLW); REE (IC); PAL[TEE/REE]	**PWS vs. N-Control:** TEE adjusted for Age/LBM/Gender =, REE adjusted for Age/LBM/Gender =, PAL ↓	The relation between TEE and REE is different in PWS children, because of reduced physical activity level.

ID	Study	Design	Sample
14	Nardella et al. (1983) [57]	Baseline results CS	**Children/Adolescents** - 12 PWS - 13 N-control (m = 15, 11-22 years)
15	Cimolin et al. (2011) [58]	O-BSD	**Adults** - 12 PWS (m = 26 years) - 19 Down syndrome (m = 29 years) - 10 N-control (m = 31 years)
16	Capodaglio et al. (2011) [59]	O-BSD	**Adults** - 14 PWS (m = 33 years) - 44 O-control (m = 34 years) - 20 N-control (m = 31 years)
17	Capodaglio et al. (2010) [60]	CCT	**Adults** - 11 PWS: 6 PWS-training, 5 PWS-control (m = 34 years) - 20 N-control
18	Cimolin et al. (2010) [61]	O-BSD	**Adults** - 19 PWS (m = 26 years) - 21 Down syndrome (m = 26 years) - 20 N-control (m = 33 years)
19	Vismara et al. (2010) [63]	Baseline results CCT	**Adults** - 11 PWS: 6 PWS-training, 5 PWS-control (m = 34 years) - 20 N-control (m = 28 years)
20	Capodaglio et al. (2009) [20]	O-BSD	**Adults** - 6 PWS (m = 27, 21-36 years) - 20 O-control (m = 29, 20-40 years) - 14 N-control (m = 30, 23-38 years)
21	Butler et al. (2007) [24]	O-BSD	**Adolescents/Adults** - 48 PWS (m = 23, 10-45 years) - 24 O-control (m =27, 11-49 years)
22	Vismara et al. (2007) [63]	O-BSD	**Adults** - 19 PWS (m = 26, 18-40 years) - 14 O-control (m = 29, 18-40 years) - 20 N-control (m = 30, 21-41 years)
23	Hakonarson et al. (1995) [64]	O-BSD	**Children/Adults** - 35 PWS (4-54 years) - Norm values
24	Greenswag (1987) [51]	CoS	**Adults** - 232 PWS (16-64 years)

Design: RCT = randomized controlled trial, CCT = controlled clinical trial, CoS = cohort study, O-BSD = observational between-subject design, CS = case series**. Sample:** m = mean age, PWS-GH = PWS patients treated with growth hormone, PWS-n-GH = PWS control group not treated with growth hormone, PWS-CoQ10 = PWS control group treated with CoQ10 supplementation, PWS-OSA = PWS patients with obstructive sleep apnea, PWS-n-OSA = PWS patients without obstructive sleep apnea, N-control = normal control group, O-control = obese control group.

Motor measurements	Main findings	Conclusions
Activity (Pedometer)	**PWS vs. N-control:** Activity =, Variability of activity ↑	Daily activity was not reduced in PWS children, but it was more variable compared with normal children.
Balance control (Force plate)	**PWS vs. N-control:** Balance control ↓, Anterior-posterior sway ↑, Medial-lateral sway ↑, Sway path ↑ **PWS vs. Down syndrome:** Anterior-posterior sway ↑, Medial-lateral sway =, Sway path ↓	PWS and Down syndrome patients have reduced balance capacity. PWS patients show poorer control in anterior posterior direction compared with Down syndrome patients.
Balance control (Force plate)	**PWS vs. O-control vs. N-control:** Balance control ↓ Anterior-posterior sway↑, Medial-lateral sway↑	PWS patients have reduced balance capacity compared with both healthy and obese individuals.
Balance control (Force plate)	**PWS vs. N-control** Balance control ↓, Anterior-posterior sway ↑, Medial-lateral sway ↑, Sway path ↑	The training program was not effective to improve balance capacity, possibly because the training was not specific.
Gait analyses	**PWS:** Forward pelvic tilt (Lordosis). **PWS vs. N-control:** Stride length ↓, Walking speed ↓, Stance phase ↑, Ankle power ↓, Ankle power/velocity = **PWS vs. Down syndrome:** Gait pattern differs because of hip stiffness in Down syndrome	Both PWS and down syndromes are characterized by abnormal gait patterns.
Gait analyses; muscle strength knee/ankle (Isokinetic dynamometer)	**PWS vs. N-control:** Cadence ↓, Stride length ↓, Velocity ↓, Stance phase ↑, Ankle power ↓, Knee flexors ↓, Ankle flexors ↓	The gait pattern of PWS patients is abnormal and muscle strength of knee and ankle flexor is reduced.
Muscle strength knee flexor (Isokinetic dynamometer)	**PWS vs. O-control, N-control:** Muscle strength knee flexor/extensor ↓, Peak torque↓, Peak torque/Weight ↓ 30%	Other factors than obesity per se seem to contribute to reduced muscular strength in PWS.
REE; TEE; EE; Physical activity (Activity energy measurement system)	**PWS vs. O-control:** TEE ↓ 20%, REE ↓ 16%, EE during specific activities ↓ 38%, EE during spontaneous activity ↓ 32%, Physical activity ↓ 35%	EE is decreased in PWS patients, because of reduced activity and lower energy utilization due to reduced lean body mass.
Gait analyses	**PWS vs. O-Control, N-control:** Cadence ↓, Single support phase ↓, Stride length ↓, Walking speed ↓, Stance phase ↑, Range of motion in knee and ankle ↓, Ankle power ↓	The gait pattern is abnormal in PWS patients, even compared with obese patients.
Pulmonary function; Thoracic muscle functioning	**PWS vs. Norm:** Forced Expiratory Volume ↓ 72%, Forced Vital Capacity ↓ 65%, Total Lung Capacity =, Thoracic muscle strength ↓	PWS patients have decreased pulmonary function primarily as a result of respiratory muscle weakness.
Motor development; Physical health; Social Emotional functioning; Impact family; Living and work (Questionnaire)	Sitting 12 months, Walking 27 months, First words 28 months, Two- to four-word phrases 35 months	PWS patients have poor gross motor skills and motor development is delayed.

M**easurements:** dev. = development, LBM = lean body mass, EE = energy expenditure, TEE = total energy expenditure, REE = resting energy expenditure, ADMR = average daily metabolic rate, BMR = basal metabolic rate, AEE = activity related energy expenditure, PAL = physical activity level. **Measurement methods:** DLW = double labeled water, IC = indirect calorimetry, BSID II = Bayley Scales of Infant Development II, WPPSI-R = Wechsler Preschool and Primary Scale of Intelligence-Revised, CCDI = Chinese Children Developmental Inventory. **Effect indication:** = = similar, ↑ = increased, ↓ = decreased, (a × b) ≠ = no correlation.

Therefore, reduced balance capacity in PWS patients could not be explained by these anthropometric parameters, or increased weight and mass and is presumably related to decreased muscle force (Table 3: 15–17). Gait analyses revealed an abnormal gait pattern in PWS adults compared with healthy controls. Walking speed, stride length, ankle power, and single support phase were decreased. Moreover, stand phase was increased and the range of knee and ankle motion was decreased (Table 3: 18, 19, 22). The deviations in the gait pattern could not be explained by increased weight and mass in PWS patients since these were not found in the obese control group (Table 3: 22).

MUSCLE STRENGTH. All studies concerning muscle strength focused on adults. Thoracic muscle strength, as measured by the maximum expiratory and inspiratory pressure, was seriously decreased. Expiratory and inspiratory pressure is normally >60 cm H_2O, but in PWS patients, expiratory pressure was below this critical point in 47% of cases and inspiratory pressure in 62% of cases (Table 3: 23). Isokinetic dynamometer measurements of the knee flexors revealed about 70% diminished muscle strength in PWS patients (Table 3: 20). Also, a decrease in muscle force of knee and ankle flexors was reported, but it was not reported how much it deviated from normal (Table 3: 19).

ACTIVITY LEVEL. Eight studies concerned activity level: one focused on infants, six on children/adolescents, and one on adults. Activity level was mostly indirectly obtained by measuring energy expenditure. Total energy expenditure was decreased in PWS infants compared with predicted values for age- and weight-matched normal reference values (Table 3: 4). In four studies, activity level in PWS children/adolescents was indirectly obtained by measuring energy expenditure, and in two studies, directly by a pedometer and/or an activity record. Basal metabolic rate or resting energy expenditure were decreased (Table 3: 8, 11–13) to 40–50% compared with normal healthy children (Table 3: 8, 11). Total energy expenditure corrected for basal metabolic rate was also decreased (Table 3: 12, 13). Contrasting results were reported for the pedometer. In the first study, PWS children/adolescents were equally active as their peers, but the variability was much higher in PWS patients (Table 3: 14), while in the second study, activity level was 50% lower compared with healthy peers and this was confirmed in the activity records (Table 3: 10). The differences between these two studies are probably related to a technically more advanced and therefore more reliable pedometer used in the second study, one that could be adjusted for stride length.

In the study with PWS adults, activity level was directly and indirectly obtained using a whole-room respiration chamber with movement sensors, during an 8-h monitoring period. Compared with obese individuals, PWS patients were not only 35% less active during the day, but their energy expenditure was also reduced during activities: spontaneous activities 32%; specific activities 38%; and in rest 16% (Table 3: 21).

PHYSICAL FITNESS. Two studies reported results on physical fitness in children/adolescents. Scores on a standardized fitness test were below the first percentile of the elementary school norms

(Table 3: 9) and exercise capacity, measured by the number of calf muscle exercise repetitions, was 31% lower than in healthy individuals (Table 3: 10).

Effects of GH treatment on motor performance in PWS

Study characteristic

A description of the study characteristics is presented in Table 4. Of the 12 included studies, six were RCTs, two CoSs and four CS. In the RCTs and CoSs, PWS patients who received GH treatment were compared with PWS patients who did not (n=6), or PWS patients who received CoQ10 supplementation (n=1), and in one RCT, the dose-dependent effect of GH treatment was examined. Six studies focused on infants, five studies on children/adolescents, and one study on adults. The study duration was 24 months or shorter except for one RCT and one CoS with a study duration of 4 and 6 years, respectively. In eight studies, the sample size was small: fewer than 20 patients were included. Six studies reported the effects of GH treatment on skill acquisition, one study on activity level, three studies on physical fitness, and two studies on both fitness and muscle strength.

Results: effects of GH treatment on motor performance in PWS

SKILL ACQUISITION. Six articles evaluated the effects of GH treatment on skill acquisition in PWS patients. All were focused on infants and standardized tests were used to evaluate motor development (Table 4: 1–4, 8, 10). However, two of these articles reported on the same RCT (Table 4: 2, 3).

Based on a RCT, Festen et al.[10] reported positive effects of GH treatment on motor development after 12 months of GH treatment. At baseline, motor development was 57% of normal reference values, and although inter-individual variation was large, infants in the GH treatment group improved by 11% compared with baseline developmental percentage, and infants in the control group deteriorated by 18% (Table 4: 1). Whitman et al.[34] reported in their RCT a positive effect of GH after six months of treatment. The treated group demonstrated a greater change in motor development compared with the control group. Also, in this study, there was a large inter-individual variation in change (Table 4: 4). In the RCT of Carrel et al.,[32] effects of GH treatment on motor development were only present in infants who started GH treatment before the age of 18 months (Table 4: 3). The GH-treated group was followed up for another 12 months and it was reported that infants receiving GH treatment walked earlier than typical PWS infants: at the age of 23.5 (Table 4: 2). These results were supported by a CS in which GH-treated infants also walked at the age of 24 months (Table 4: 10) after 12 months of GH treatment. However, in the CoS in which GH treatment was compared with CoQ10 supplementation, no effect of GH treatment on motor development was found: both groups improved equally after 12 months of treatment. According to the authors, the improvement may reflect an age-related phenomenon additionally depending on early diagnosis and introduction of appropriate care (Table 4: 8).

Table 4. Characteristics of randomized GH effect studies

ID	Study	Design	Sample	Intervention
1	Festen et al. (2008) [10]	RCT	**Infants** - 16 PWS-GH - 18 PWS-n-GH (m = 2, 1-3 years)	GH vs. n-GH 12 months
2	Myers et al. (2007) [53] *Suppl. Carrel et al. (2004)*[32]	RCT	**Infants** - 14 PWS-GH - 11 PWS-n-GH (m = 15, 4-37 months)	GH 24 months vs. n-GH 12 months and 12 months GH high dose
3	Carrel et al. (2004) [32]	RCT	**Infants** - 15 PWS-GH - 14 PWS-n-GH (m = 15, 4-37 months)	GH vs. n-GH 12 months
4	Whitman et al. (2004) [34]	RCT	**Infants** - 18 PWS-GH - 12 PWS-n-GH (m = 16, 4-45 months)	GH vs. n-GH 6 months
5	Carrel et al. (2002) [29] *Suppl. Carrel et al. (1999)* [38]	RCT	**Children** - 14 PWS-GH-0.3 - 18 PWS-GH-1.0 - 14 PWS-GH-1.5 (m = 11, 5-16 years)	GH 24 months and GH dosage 24 months
6	Carrel et al. (1999) [38]	RCT	**Children** - 35 PWS-GH - 19 PWS-n-GH (m = 10, 4-16 years)	GH vs. n-GH 12 months
7	Carrel et al. (2010) [52]	CoS	**Children** - 21 PWS-GH (m = 7, 6-9 years) - 27 PWS-n-GH (m = 8, 5-9 years)	GH 6 years vs. n-GH
8	Eiholzer et al. (2008) [9]	CoS	**Infants** - 6 PWS-GH, Weight < 0 SDS (m = 0.7 years) - 12 PWS-GH, Weight = 0 SDS (m = 1.3 years) - 8 PWS- CoQ10, Weight = 0 SDS (m = 0.6 years)	GH vs. CoQ10 12 months
9	Gondoni et al. (2008) [65]	CS	**Adults** - 12 PWS-GH (m = 26, 20-33 years)	GH 12 months
10	Eiholzer et al. (2001) [8]	CS	**Infants** - 10 PWS-GH (m = 1, 0-4 years) - Normal values	GH 4 years
11	Myers et al. (2000) [56] *Suppl. Carrel et al. (1999)* [38]	CS	**Children/ Adolescents** - 35 PWS-GH (m = 10, 4-16 years)	GH 24 months
12	Eiholzer et al. (1998) [40]	CS	**Children/Adolescents** - 3 PWS-under-W-GH (0-4 years) - 6 PWS-over-W-GH (3-7 years) - 3 PWS-adolescents (9-16 years)	GH 12 months

Design: RCT = randomized controlled trial, CoS = cohort study, CS = case series. **Population:** m = mean age, PWS-GH = PWS patients treated with growth hormone, PWS-n-GH = PWS control group not treated with growth hormone, PWS-CoQ10 = PWS control group treated with CoQ10 supplementation.

Motor measurements	Main findings	Conclusions
Metal dev.; Motor dev. (BSID II)	**T0** GH vs. n-GH: Mental dev. = 72%, Motor dev. = 57%. **T0 vs. T.12mo** - GH vs. n-GH: Mental dev. ↑, Motor dev. ↑	GH treatment improved mental and motor development.
Motor dev. (TIME); Language dev. (Questionnaire)	Results T0, T.12mo Carrel [32] **T.12mo vs. T.24mo** PWS-GH: First words 14 mo, Walking 23.5 mo	Motor and language development improved during GH treatment.
Motor dev. (TIME)	**T0** - GH vs. n-GH: Motor dev. = **T0 vs. T.12mo** - GH vs. n-GH: Subgroup < 18 mo: Motor dev. ↑	When GH treatment starts before 18 months of age it increases mobility, and skill acquisition.
Motor dev. (TIME)	**T0 vs. T.6mo** - GH vs. n-GH: Motor dev. ↑	GH treatment improved motor development.
REE (IC, n=26); Strength/agility (Bruininks Oseretsky test)	**T.24m vs. T.4y** - In all dosage groups, strength/agility stabilized	GH-induced changes in physical functioning can be sustained for 48 months.
REE (IC); Strength/ agility (Bruininks Oseretsky test); Thoracic muscle strength	**T0** - PWS vs. normal: REE ↓ **T0 vs. T.12mo** - GH: REE =, Strength/agility ↑, Running speed ↑, Broad jumping ↑, Sit-ups ↑, Arm curls ↑, Thoracic muscle strength ↑	GH treatment improved physical functioning and thoracic muscle strength.
Strength/agility (Bruininks Oseretsky test)	**PWS-GH vs. PWS-n-GH** Strength/agility ↑, Broad jump ↑, Sit-ups ↑	When GH treatment starts before 24 months of age it improves motor function.
Psychomotor dev. (Griffith test)	**T0** - PWS vs. Norm: Global dev. ↓ 54-66%, Motor dev. ↓ 30-44% - GH vs. CoQ10: Global dev. =, Motor dev. = **T0 vs. T.12mo** - GH vs. CoQ10: Global dev. =, Motor dev. = - GH: Global dev. ↑, Motor dev. ↑ - CoQ10: Global dev. ↑, Motor dev. ↑	Improved psychomotor development, did not differ between GH and CoQ10 treated infants.
Exercise capacity (Treadmill test)	**T0 vs. T.6mo, T.12mo** Exercise capacity ↑	GH has beneficial effects on physical activity and agility.
Psychomotor dev. (Griffith test)	**T0 vs. T.12 mo** Motor dev ↑, Walking 24 mo	GH treatment may enhance motor development.
REE (IC, n=16); Strength/ agility (Bruininks Oseretsky test); Thoracic muscle strength (n=20)	**T0 vs. T.12mo** REE ↑, Strength/agility ↑, Running speed ↑, Broad jumping ↑, Sit-ups ↑, Arm curls ↑, Thoracic muscle strength ↑ **T.12mo vs. T.24mo** REE =, Strength/agility ↑, Running speed =, Broad jumping ↑, Sit-ups ↑, Arm curls =, Thoracic muscle strength =	GH treatment improved physical functioning, and thoracic muscle strength.
Physical activity (Interview)	**T0 vs. T.12mo** Parents reported increased physical activity	GH improved physical performance.

Measurements: *dev. = development, REE = resting energy expenditure.* ***Measurement methods:*** *IC = indirect calorimetry, BSID II = Bayley Scales of Infant Development II, TIME = Toddler and Infant Motor Evaluation.* ***Effect indication:*** *= = similar, ↑ = increased, ↓ = decreased.* ***Findings:*** *T0 = baseline results, T.xmo = results trail x months.*

MUSCLE STRENGTH. In two studies, the effects of GH treatment on muscle strength in PWS children/adolescents were reported (Table 4: 6, 11). In both studies, thoracic muscle strength was evaluated by measuring maximum expiratory and inspiratory pressure. In the RCT, thoracic muscle strength improved in the GH treatment group: expiratory muscle force about 27%, and inspiratory muscle force about 22% (Table 4: 6). This finding was supported by a two-year CS. In the first treatment year, thoracic muscle strength improved, and in the second year, it remained stable (Table 4: 11).

ACTIVITY LEVEL. In one CS, parents of children/adolescents reported improvements in activity level after 12 months of GH treatment (Table 4: 12).

PHYSICAL FITNESS. The effect of GH treatment on physical fitness was evaluated in five studies: four studies focused on children/adolescents (Table 4: 5–7, 11), and one study on adults (Table 4: 9). In children, fitness was evaluated using a modified standardized motor test containing running speed, broad jumping, sit-ups, and arm-lifts. One RCT reported positive effects of 12 months of GH treatment on physical fitness. After one year of treatment, patients ran 2.3 seconds faster, jumped 3.3 inches further, and did three more sit-ups/20 seconds and 2.5 more arm-lifts/ 30 seconds compared with baseline. Fitness did not improve in the control group (Table 4: 6). This treatment group was followed for another treatment year in a CS and running and arm-lifts further improved (Table 4: 11). In a CoS, patients who received GH treatment for six years were compared with patients naïve to GH. GH-treated children jumped 8 inches further, and did four more sit-ups (Table 4: 7). In an RCT focusing on the dose-dependent effect of GH treatment, it was reported that the initial improvement in fitness, as measured after 24 months full-dose treatment, was sustained for another two years in all GH-dosage groups, including the low-dose group (Table 4: 5).

In PWS adults, physical fitness was evaluated in a CS using a treadmill test. After 12 months of GH treatment, exercise duration improved from 8.8 to 10.5 minutes and exercise intensity, expressed as metabolic equivalents, improved by 19% (Table 4: 9).

Physical training in PWS

Study characteristics

A description of the study characteristics is presented in Table 5. Ten studies were included: five CCTs, and five CS. Four studies focused on children/adolescents, and six studies on adults. In all studies except one, the sample size was fewer than 20 PWS patients. There were three types of physical training programs: exercise programs containing specific muscle strength exercises; activity programs containing activities such as walking, swimming, etc.; and a combination of both. Physical training was frequently combined with a diet. Most physical training programs were carried out during residential care. The duration of the physical training programs varied considerably from two weeks to one year. All physical training programs had a sub-maximal intensity and most of them were performed on a daily basis. The studies intended to assess the

effects of physical training on activity level, physical fitness, weight, body composition, or a combination of these.

Results: physical training in PWS

It is not possible to relate the PWS physical training studies to each other, since the content of the physical training programs, the intended effects, and the measurement instruments differed considerably between the studies. As all studies reported positive effects from physical training, it is interesting to see what the content of the physical training was and what the results were. In the CS of Grolla et al.[66], a physical training program for weight management in PWS adults in a residential care setting was evaluated and improved. The program contained 60 minutes of step and cycling exercises, 60 minutes of mat exercises, and a total of three hours of walking per day. In the initial 14-day program, compliance decreased and temper tantrums increased in the second week. The program was improved: activity and rest periods were balanced; the duration was prolonged to 21 days; and entertainment activities were added. In the improved program, compliance decreased and weight loss stabilized during the third week. In the final program, two hours of psychomotor and music therapy were added, and the duration was extended to four weeks. Compliance and weight management were most successful in the final program, especially when the patients participated in the program three times a year in succession. The authors remarked that: (1) activities were performed at a lower speed than normal and with frequent interruptions; (2) groups of more than 10 patients would be hard to manage, due to the complicated social dynamics; (3) teamwork and competition are important factors for motivation; (4) psychomotor and music therapy was especially appreciated by the patients; and (5) excessive fatigue must be avoided since this led to temper tantrums and refusal to cooperate (Table 5: 1). In the CCT of Capodaglio et al.[60], a physical exercise program for PWS adults to improve balance capacity was evaluated. The program was introduced during a two-week hospital stay in which the patients participated in supervised exercise sessions. Four times a week, patients had to perform four exercises to improve ankle and knee muscle strength. After the initial two-week period, the physical training group continued the exercise program at home for six months. During the hospital stay, in which the whole group participated in the program, balance capacity did not improve in both groups and also after six months there was no improvement. The authors reported that training exercises were aimed at ankle and knee muscle strength and that balance and proprioceptive exercises would possibly be more effective to improve balance capacity (Table 5: 2). The same research group studied in a CCT a physical exercise program for PWS adults to improve gait. The training program and exercises were similar to the training program to improve balance capacity. During the hospital stay in which the whole group participated the program, knee and ankle function during gait improved in both groups. After six months, cadence increased, and ankle and knee function further improved in the physical training group and no change was seen in the control group (Table 5: 3).

Table 5. Physical training in PWS

ID	Study	Design	Sample	Intervention
1	Grolla et al. (2011) [66]	**CS**	**Adolescents/Adults** - 49 PWS (m = 24, 13-42 years)	**Training program + diet** (30 days, 4 times a year, daily 6.5 hours: Muscle-strength-exercises/Cycling/Walking/ Psychomotor-therapy/Music-therapy)
2	Capodaglio et al. (2010) [60]	**CCT**	**Adults** - 11 PWS: 6 PWS-training, 5 PWS-control (m = 34 years) - 20 N-control (m = 31 years)	**Exercise program** (6 months, 4 times a week, 30 minutes: Muscle-strength-exercises)
3	Vismara et al. (2010) [62]	**CCT**	**Adults** - 11 PWS: 6 PWS-training, 5 PWS-control (m = 34 years) - 20 N-control (m = 28 years)	**Exercise program** (6 months, 4 times a week, 30 minutes: Muscle-strength-exercises)
4	Schlumpf et al. (2006) [45]	**CCT**	**Children** - 7 PWS-training - 12 PWS-control (m = 9, 6-12 years)	**Exercise program** (6 months, daily 4-10 minutes: Muscle-strength-exercises)
5	Eiholzer et al. (2003) [44]	**CCT**	**Children/Adolescents** - 17 PWS (m = 10, 4-19 years) - 18 N-control (m = 11, 5-20 years)	**Exercise program** (3 months, daily 3-4 minutes: Muscle-strength-exercises)
6	Kaufman et al. (1995) [67]	**CS**	**Adults** - 8 PWS (17-32 years)	**Exercise program + diet** (1 year, 3 times a week, 30 minutes: Muscle-strength-exercises)
7	Silverthorn and Hornak (1993)[46]	**CCT**	**Adults** - 6 PWS-training - 5 PWS-control (m = 28 years)	**Activity program** (6 months, 2-4 times a week: Walking 2.5 - 10 km.)
8	Mullins and Vogl-Maier (1987) [30]	**CS**	**Children** - 8 PWS (8-13 years)	**Training program + diet** (26-days, daily: Muscle-strength-exercises/Walking/ Swimming)

Motor Measurements	Main findings	Conclusions
W; Training compliance	**Training 14 days** - W ↓ 0.19 kg daily, Compliance ↓, Temper tantrums ↑ **Training 21 days** more rest periods + entertainment. - W ↓ 0.22 kg daily, Compliance ↓ **Training 28 days** psychomotor + music therapy. - W ↓ 0.26 kg daily, Compliance =	Weight loss is achievable in PWS adults by participating in a 28-day training program 3 times a year. in which activity, rest periods, and entertainment are balanced.
Balance control (Force plate)	**PWS vs. N-control** Balance control ↓, Anterior-posterior sway ↑, Medial-lateral sway ↑, Sway path ↑ **T0 vs. T.2 weeks** training in all patients. - = **T2 weeks vs. T.6mo** PWS-training: = PWS-control: =	The training program was not effective to improve balance capacity, possibly because the training was not specific.
Gait analyses; Muscle strength knee/ankle (Isokinetic dynamometer)	**PWS vs. N-control** - Cadence ↓, Stride length ↓, Velocity ↓, Stance phase ↑, Ankle power ↓, Knee flexors ↓, Ankle flexors ↓ **T0 vs. T.2 weeks** training in all patients. - Ankle/knee function ↑ **T2 weeks vs. T.6mo** PWS-training: Cadence ↑, Ankle/knee function ↑ PWS-control: =	PWS adults are able to follow a simple home training program which effectively improves physical functioning.
LBM (DEXA); Walking distance (Pedometer); Activity record	**T0 vs. T.6mo:** - Training: LBM ↑ - Control: LBM = - Training vs. Control: LBM ↑, Walking ↑, Activity ↑	Children with PWS can be motivated by their families to follow a daily training program, which has effects on physical activity and increase, but not normalize LBM.
Walking distance (Pedometer); Activity record; Exercises repetitions	**T0** - PWS vs. N-Control: Walking ↓ 50%, Activity ↓, Exercises ↓ **T0 vs. T.3mo** Training. - PWS: Walking ↑, Activity =, Exercises ↑ - N-Control: Walking =, Activity =, Exercises ↑ **T0 vs. T.6mo** Follow-up. PWS: Exercises ↑ N-control: Exercises ↑	An easy-to-accomplish training program improves local body composition in PWS patients and has generalized effects on physical activity and capacity.
W; Training compliance	**T0 vs. T.1y** W ↓, Training compliance 60%	Controlling weight in PWS patients can be highly effective in specialized group homes.
W; RHR; Aerobic capacity (Cycling test); Walking distance; Walking speed	**T0 vs. T.6mo:** - PWS-Training: W ↓, RHR ↓, Aerobic capacity ↑, Walking distance ↑ 3.7 – 10 km., Walking speed ↑ 21.5 – 15 min/km. - PWS-Control: W =, RHR ↑	Training can make a significant contribution to weight loss, and increased aerobic capacity in adults with PWS.
W; Relative W ((W - Ideal W)/Ideal W); Cardiovascular fitness; Pulse rate, 1-minute sit-up test; Shuttle run test	**T0 vs. T.26day:** Pulse rates ↓, Sit-ups ↑, Shuttle run test = below the first percentile. **Over 3 years:** Relative W ↓	It is possible to increased physical fitness in PWS children.

ID	Study	Design	Sample	Intervention
9	Caldwell et al. (1986) [68]	**CS**	**Adults** - 11 PWS m = 21, (14-32 years)	**Activity program + diet** (5 weeks, daily 30 minutes: Walking/ Swimming/ Aerobic-dancing/Cycling)
10	Nardella et al. (1983) [57]	**CS**	**Children/Adolescents** - 12 PWS - 13 N-control (m = 15, 11-22 years)	**Training program + diet** (2 weeks, daily: Muscle-strength-exercises/Walking)

Design: CCT = controlled clinical trial, CS = case series. **Population:** m = mean age. M**easurements**: W = weight, LBM = lean body mass, RHR = resting heart rate. **Measurement methods:** DEXA = dual energy X-ray absorptiometry.

In the CCT of Schlumpf et al.[45], a home physical exercise program for PWS children was evaluated. The 6-month program contained daily exercises of 4–10 minutes for abdominal, breast, arm, shoulder, and leg muscles to improve lean body mass and activity level. Lean body mass was evaluated by measuring body composition, and activity level was evaluated using a pedometer and activity records. Lean body mass increased in the physical training group (from −1.83 to −1.49 standard deviation score). Since the physical training group already had received long-term GH treatment, the authors concluded that physical training had an additional effect on body composition. Although spontaneous activity increased according to the pedometer (from 12.6 to 14.1 km per 3 days), it remained stable, as reported in the activity records. In the PWS patients in the control group, all parameters remained stable (Table 5: 4).

A 3-months home physical exercise program was evaluated in the CCT of Eiholzer et al.[44] In PWS patients and healthy control children, daily calf exercises were carried out for 3–4 minutes to improve calf muscle mass, activity level, and physical fitness (exercise capacity). Calf circumference and calf skinfold were taken to evaluate muscle mass. Activity level was evaluated by a pedometer and activity records, and exercise capacity by the amount of calf exercise repetitions. Muscle mass improved, since calf circumference increased and calf skinfold decreased. Although activity level increased based on the pedometer (from 11.1 km to 17.4 km measured over 3 days), it remained stable as reported in the activity records. The amount of calf exercise repetitions improved (from 22.7 to 57.3). After three months of follow-up, the increase in muscle mass disappeared in healthy children, but it remained in PWS patients (Table 5: 5).

A physical exercise and diet program for PWS adults was performed in a residential care setting in the CS of Kaufman et al.[67] During one year, patients took 50% of normal caloric intake and performed 30 min of exercises for three days a week to lose weight. On average, weight decreased by 17 kg. However, the authors concluded that physical exercise may not have been a

Motor measurements	Main findings	Conclusions
Exercise units (30 minutes Walking/ Swimming/Aerobics/ Cycling)	n=3. No reinforcement, vs. Low preference food vs. High preference food: Exercise = n=1. No reinforcement vs. Food reinforcement: Exercise ↑, High vs. low preference food: Exercise = n=5. No reinforcement vs. Low preference food: Exercise =, Low preference food vs. High preference food: Exercise ↑ n=2. No reinforcement vs. Low preference food: Exercise ↑, Low preference food vs. High preference food: Exercise ↑	Food can be used to reinforce activity and exercise in PWS patients.
W; Activity (Pedometer)	**T0** - PWS: vs. N-control: variability of Activity ↑ **T0 vs. T.2wk** - PWS: W ↓, (Weight loss × Activity) ≠	Self-motivation and positive reinforcement may be a powerful influence on the physical activity level of certain PWS patients.

Effect indication: = = similar, ↑ = increased, ↓ = decreased, (a × c) ≠ = no correlation. **Findings:** T0 = baseline results, T.xmo = results trail x months.

large contributor to the weight loss, since training compliance was only 60% and the intensity of training was probably not sufficient (Table 5: 6).

In the CCT of Silverthorn and Hornak,[46] an activity program for PWS adults was evaluated in a residential care setting. During six months, the physical training group walked 2.4–10 km, 2–4 times a week to decrease body fat percentage, weight, and improve physical fitness. Skinfold measurements were taken to determine body fat percentage, and physical fitness was assessed by resting heart rate and maximum oxygen uptake during a cycling test. Upper arm body fat decreased (from 8.6 to 5.46 mm), weight decreased (from 54.6 to 49.43 kg), resting heart rate decreased (from 61 to 56 beats per minute), aerobic capacity improved (from 1.96 to 2.25 volume of oxygen in liters per minute). Furthermore, walking speed improved from 20–23 min/km to 13.5–16.5 min/km. In the control group, weight, skinfolds, and aerobic capacity remained stable, and resting heart rate increased. Walking speed was not evaluated in the control group (Table 5: 7).

In the CS of Mullins and Vogl-Maier,[30] a diet and physical training program for PWS children in a summer camp setting was evaluated. During 26 days, patients performed a daily individual exercise routine, 45 minutes walking twice a day, and five hours swimming each week to improve weight and physical fitness. Physical fitness was evaluated by measuring pulse rate, after walking one kilometer, the amount of sit-up repetitions, and by a standardized fitness test. Pulse rate decreased and the amount of sit-ups increased. The scores on the standardized fitness test were below the first percentile of elementary school norms both before and after the physical training program. In children who participated in the program for three summers in succession, relative weight decreased, while it normally increases in PWS children (Table 5: 8).

Caldwell et al.[68] evaluated, in a residential care setting, whether food can be used to reinforce activity in PWS adults. During five weeks, patients were on a diet and could 'earn' food by performing activity units: 30 min of walking, swimming, aerobics, or cycling. It was concluded

that food can be used as a reinforcer for increased activity, because there seemed to be an effect in some patients, although these finding were not statistically confirmed (Table 5: 9).
In the CS of Nardella et al.,[57] a diet and physical training program in a summer camp setting for PWS children was used to improve weight and activity level. During two weeks, patients performed a daily exercise routine and took a daily walk. Weight decreased on average by 3.6 kg, there was no correlation between activity level and weight. Changes in activity level were not further discussed in this article (Table 5: 10).

Discussion

Principal findings

The first goal of this literature study was to gain insight into motor performance in PWS patients. Motor development is seriously affected in PWS infants.[8-10,50,54,55] Scores on standardized motor developmental tests were 30–57% of the normal reference values,[8-10,54,55] and in children and adults, significant motor problems were reported in all four domains: (1) balance control is decreased,[58,59] gross motor skills such as walking,[61-63] running, and jumping are impaired;[38,56] (2) muscle strength is decreased;[20,64] (3) activity level is lower;[24,25,28,38,44,56,57] and (4) physical fitness is decreased.[30,44] The second goal was to evaluate the effects of GH treatment on motor performance in PWS patients. All RCTs reported a significant positive effect of GH treatment on motor development in infants,[10,32,34] especially if the intervention started before the age of 18 months.[32] However, in the CoS of Eiholzer et al.,[9] no significant effects were demonstrated. In children, GH treatment was associated with small improvements in muscle strength and physical fitness in the first treatment year.[38,52,56] In adults, the one performed study reported small effects of GH treatment on physical fitness.[65] The third goal of this study was to analyze the effects of physical training programs in PWS patients. Most physical training programs were executed during residential care, focusing on strengths and/or fitness, and nine of the 10 studies reported beneficial effects in children and adolescents on body weight, body composition, activity level, physical fitness, or a combination of these.[30,44-46,62,66,67] In the study that reported no training effects, ankle and knee muscle exercises were used to improve balance capacity.[60] It was strange that the authors hypothesized such an effect from simple intervention, because we know from literature that muscle force is only one of the factors influencing balance, therefore functional training would have been more appropriate.[69-71] The same exercise program did have a positive effect on gait.[62] The reason for this is that walking is a daily functional activity, so muscle force gain was immediately integrated in daily skills.

Study characteristics

The sample size of the included studies was relatively small, probably due to the low incidence of the syndrome. This is presumably also the reason why the age range of the included patients was sometimes quite large. The studies concerning motor performance and the GH treatment effect studies in PWS were mostly methodologically well performed. The studies concerning physical

training in PWS varied from good to moderate. In all three topics, there was little overlap in outcome variables between studies and therefore a meta-analysis was not possible.

Motor performance in PWS

Although the motor developmental studies do provide some insight into the ICF activity level, it is not clear to what extent the quality of particular motor skills is affected and what consequences this has on the sequence of motor milestones and general psychomotor development. Especially in the early years, PWS infants are unable to move against gravitational forces, because of severe hypotonia and muscle weakness.[6,7] Infants are not able to turn their head, reach, grasp, roll over, or sit upright, which hampers their ability to discover the world around them. In the clinical practice, parents report difficulties in handling, feeding, and interacting with their child. Since feeding takes more time and is exhausting for the infant, there is little time and energy left to play and interact with the child. Also, since motor development is seriously delayed, the opportunities to play and interact with the child are limited. In fact the developmental profile of PWS infants is found to be disharmonic.[55] Gross motor development, eye-hand coordination, and speech are more affected than social development and IQ.[8,9] However, cognitive problems become more prominent over time. This could be the effect of motor delay negatively influencing other developmental domains, since each new motor skill opens up opportunities for perceptual, motor, and social explorations, allowing the infant to interact with and learn from its environment. Moreover, although mental development is less affected than motor development in infancy, they are correlated.[10] This is probably due to the fact that in most cognitive tasks, especially in infants, motor skills are vital. Furthermore, since major brain developmental processes take place during the first few years of life,[72-74] and brain development is guided by an ongoing interaction between the organism and the environment,[75] motor activity is a strong stimulator for structural and functional brain development in infancy.[76-78] Because of the hypotonia, muscle weakness, and motor developmental delay, the natural environment does not automatically provide a good learning opportunities for PWS infants. We hypothesize that it is possible to stimulate psychomotor development in PWS infants by providing the parents and other caregivers with adequate information and by educating the parents how they can provide learning conditions for their child by manipulating the environment and attune it to the abilities of the child. Some evidence was found that the effect of GH treatment on motor development is stronger when treatment is started before 18 months of age.[32] This is typically the age when muscle force is needed to overcome the forces of gravity. Another finding in the studies was the inter- and intra-individual variability of motor development.[10,34] The studies did not provide insight into whether or not the variability was related to genetic differences, differences in body function and structure, or differences in contextual factors. Moreover, it is not clear if motor performance level at a younger age is predictive for the progress of motor development and performance and activity level at an older age. Moreover, we do not have insight in the interaction between the motor, cognitive and behavioral domains over time. In adulthood, most

patients function in the "mild retardation range" and show a specific profile of cognitive strengths and weaknesses. For example, short-term memory is more affected than long-term memory,[79,80] deficits in auditory processing are more pronounced than in visual processing[81,82] and behavioral problems like obsessive-compulsive disorders are more prominent.[83] To answer these questions, longitudinal studies would be necessary in which development is frequently evaluated in a detailed manner, taking into account differential interacting factors.

In PWS children and adults, it is clear that there are problems at all of the ICF levels. At the activity level, gross motor skills such as walking, running, and jumping are impaired.[38,52,61,63] At the level of body structure and function, PWS patients have abnormal body composition, decreased muscle mass and strength, and lower physical fitness.[20,44,56,67] Because of the decreased activity level, the participation in sports, leisure and work is affected as well.[25,28,38,56] Moreover, since basic motor activities such as balance control and gait are affected in PWS patients, it is likely that other gross motor skills are affected as well and that this hampers normal daily activities. These abnormalities are presumably related to decreased muscle force, decreased coordination, and decreased daily activity. Therefore, it would be interesting to study motor impairment in PWS adults in a more consistent way, connecting impairments in structure and function, activity, and participation levels to each other.

Despite the fact that decreased muscle strength is a major life-long characteristic of PWS, there are only two studies focusing on this topic.[20,64] In the literature, there is discussion as to whether decreased muscle strength in PWS patients is solely the result of decreased muscle mass or that the quality of the muscle itself is also a determining factor.[23,84] In this perspective, it would be interesting to study the relationship between decreased muscle mass, decreased muscle strength, the structure of the muscle, and the effect of GH treatment, physical training, or the combination of both GH treatment and physical training. Moreover, the muscle impairments will have consequences on skill acquisition and physical endurance, which influences the normal motor skills needed in daily life.

The results concerning activity level were not consistent. Because activity level was mostly measured indirectly by evaluating energy expenditure, there was a difference in the interpretation of the findings between authors. Some studies report that activity level is normal since decreased energy expenditure could be explained by the fact that metabolic rate is decreased as result of lower lean body mass in PWS.[31,85] Other studies reported that even when correcting for decreased lean body mass, energy expenditure is still decreased, and interpreted this as the result of a lower activity level.[24-28] It still remains unknown whether the decreased energy expenditure is related to a lower activity level during the day, or to a lower intensity of activities or even to an abnormal metabolism. Butler et al.[24] tried to tackle the problem, by measuring energy expenditure in a whole-room respiration chamber with movement sensors during an eight-hour monitoring period. They reported that energy expenditure, corrected for decreased metabolic rate, was reduced during spontaneous and specific activities, and that PWS patients were 35% less active during the day.[24] Hence, the problem seems to be more complex

and it should be evaluated whether PWS patients have a decreased intensity level because of motor problems, decreased cardiovascular fitness, or a different metabolic level. Finally, it would be interesting to evaluate activity level in PWS related to the genetic subtypes, IQ, and social emotional well being.

Concerning physical fitness, two studies reported decreased physical fitness in PWS.[30,44] Since heart rate or $V_{O2\ max}$ was not measured in these studies, it is not possible to determine whether the lower level of physical fitness was constrained by poor motor performance, decreased cardiovascular fitness, or decreased motivation.

Effects of GH treatment on motor performance in PWS

Due to the large inter-individual variation in motor performance between PWS infants and children, the small number of participants in the studies, the large variation in the age at which GH treatment was started, and the differences in dose and duration of treatment, it is difficult to demonstrate beneficial effects of GH treatment on motor performance.

In infants, the variation in developmental level between subjects is large at the start of the intervention.[10,34] These differences are presumably related to differences in genotype, health status, feeding problems, growth parameters, cognition, and life circumstances. These factors should be taken into account when studying the effects of GH treatment on motor development in PWS infants. Moreover, the control periods of RCTs and CoSs are relatively short for determining long-term effects. Considering these methodological difficulties, it is promising that in a few RCTs, positive effects of GH treatment on motor development were demonstrated.[10,32,34]

It would be interesting to evaluate the relationship between the beneficial effects of GH treatment on muscle mass and body composition and the increase in motor development and skill acquisition to gain insight into the working mechanism. Therefore, it would be recommendable to evaluate the progress in motor development longitudinally in more detail, more frequently, and in relation to body structure and function. Moreover, because of the low incidence of PWS, it would be interesting to develop an international study protocol in a multidisciplinary perspective.

Physical training in PWS

It is promising that physical training seems to lead to improved motor performance and health status in PWS patients. From the results, we can conclude that it is possible and beneficial to physically train PWS patients. However, the physical training programs evaluated in the included studies were mostly quite general, and not meaningful and motivating to the PWS individual. For example, in all studies except one,[62] muscle strength exercises in the physical training programs were not functionally connected to improvement of particular motor skills for daily life.

Moreover, the effect of physical training is highly determined by intensity and duration of the training and the adherence over a longer time.[43,66,86] This means that the training level should be determined for each individual separately to increase skill level, muscle force, and cardiovascular

fitness. We hypothesize that PWS patients could benefit far more from physical training when the program is focused on specific individual goals, tuned to individual needs, and connected to daily activities. Physical training should focus on specific barriers in motor skill level that hinder PWS patients from participating in particular sports, outdoor activities, daily life activities, or work. As stated by Grolla et al.,[66] it is necessary to search for conditions that intrinsically motivate the PWS individual to train, to move, and to enjoy this, which increases training compliance. However, none of the tested programs focused on such forms of skill acquisition.

Since decreased muscle strength is a characteristic of PWS, a specific muscle-force training program should be part of the program to improve muscle strength necessary to learn new motor skills. In one program, we found that ankle and knee muscle strength was effectively trained to improve gait.[62] Although, muscle force training on a young age could potentially prevent or reduce scoliosis, which is frequently seen in PWS patients,[2] none of the studies focused on the effect of training on scoliosis. A really important point is the focus on cardiovascular fitness and weight loss in PWS patients. There were a few physical training programs in which weight loss and improvement of physical fitness was intended. However, both goals ask for different approaches which should be combined. For weight loss, it is important to train at a sub-maximal level with appropriate duration, activities such as walking, cycling, and swimming can be used, but to improve cardiovascular fitness, training incentive has to be high enough.[43] The physical training programs used did not fulfill these criteria.

A more remarkable fact was that we did not find any information about interventions in infants, especially since, in the clinical practice, parents have a lot of questions on how to handle their child with severe hypotonia and seriously delayed motor development.[8-10,50,54] In addition, as stated before, in infancy, motor development is conditional for other developmental domains, since it influences the opportunities to interact with and learn from the environment. Therefore, especially in this early period of life, there should be a strong focus on stimulating motor and neuromuscular development.[76-78] In our opinion, physical training could be a powerful tool to achieve this. Since training should be incorporated in daily life in order to be effective, it is important to involve the parents or care takers. In normal developing infants, their environment gives them almost unlimited excess to social, cognitive and motor explorations,[87] which is presumed to optimize their brain development.[88,89] In PWS infants the environment is not automatically attuned to their abilities. A specialized pediatric physical therapist is needed to coach the parents in realizing a learning environment to stimulate development of their child and to help them with interactive playing, attuned to the abilities of their child.

General conclusions

The fourth goal was to formulate advices for intervention and research programs to improve motor performance, activity level, and participation in PWS patients. The most important topic for professionals, parents, and caregivers is whether the child is physically healthy, emotionally well, and growing up to their potential. However, to be able to provide evidence based advices,

we detected several gaps in the actual knowledge in motor performance in Prader–Willi Syndrome. We will start by formulating the conclusions on future research topics, after which we will, based on the actual state-of-the-art in literature, draw a number of conclusions on the clinical management of motor performance problems.

Conclusions on research topics

The implementation of complex interventions to improve healthcare goes through particular phases.[90] The Medical Research Council advised a preclinical theoretical phase in which relevant theories are explored to formulate hypotheses. Then in clinical phase I the components of the intervention and the proposed underlying mechanisms are identified. In clinical phase II the intervention is described in a replicable way and tested in a pilot study where it is compared to an appropriate alternative. In clinical phase III the fully defined intervention is tested on the hypothesized outcomes in a Randomized Clinical Trial with appropriate power (gold standard for evidence), using a protocol that is theoretically defensible, reproducible, and adequately controlled. In clinical phase IV implementation of an effective intervention is tested, determining whether others can reliably replicate the intervention and results.[90] Although it is useful to think in terms of phases, in practice these may not follow a linear or even a cyclical sequence.[91] Looking to the results of this review we have to conclude that there are gaps in the necessary knowledge in the development of interventions to improve motor performance in PWS patients. For instance GH is implemented in clinical practice (clinical phase IV), while the training interventions are mostly only tested in pilot studies or small controlled trials (clinical phase I or II). However, for both GH and physical training there are still very important fundamental theoretical questions that need to be addressed. Firstly, studies should focus on the causes of muscle weakness and hypotonia in neuromuscular structures and/or neurometabolic functions.[23,84,92,93] This is required to understand the underlying working mechanism of GH and physical training. Secondly, studying the relationship between physical and behavioral features and the genetic imprinting would be an important topic as this would increase understanding of why some patients profit more from GH or physical training than others. PWS animal models could be useful in these sort of studies.[94] Thirdly, although PWS patients seem to profit equally as healthy individuals from training,[44-46] it is important to further explore the effectiveness of specific training approaches (skill training, muscle force training, and low or high intensity cardiovascular fitness training) with respect to the abnormal body composition, muscle functioning, and neurometabolic functioning in PWS. In this light also the relationship between training and necessary adaptation in food intake should be studied. Another large gap in our knowledge is a lack of insight in longitudinal development, not only concerning motor performance but also in relation to cognitive and behavioral development. These are important questions from parents and caretakers of PWS patients. Therefore, we advocate longitudinal multidisciplinary studies focusing on all phases as presented by the MRC. Because of the low

incidence of the syndrome, we strongly recommend international cooperation and study protocols.

Conclusions on clinical practice

The care for PWS patients is recommended to be a multidisciplinary approach with a pediatrician, pediatric endocrinologist, neurologist, pediatric physical therapist, dietician, speech therapist, and psychologist and psychiatrist.[1,17] In this conclusion we focus especially on motor performance with a role for the pediatric physical therapist in training. We conclude that physical activity and muscle force training need to be an important part of daily life in PWS patients of all ages. The more so, because muscle mass improvement, as a result of training, sustained.[44] More importantly, the muscle force improvement in PWS patient is functionally used in daily physical activities, otherwise it would have faded away after finishing training, as it did in healthy control children.[44] During the growth to adulthood physical training should be focused on: (1) stimulating psychomotor development; (2) improvement of particular motor skills and pleasure in physical activity, which is needed to improve activity level in long-term and participation in sports and outdoor activities; and (3) improvement of cardiovascular fitness and endurance, which is needed to control weight and to maintain a healthy condition. Physical activity can only become part of daily life when someone is intrinsically motivated and experiences pleasure. Therefore, we advise to participate in relatively short physical training sessions, combined with coaching programs to increase daily physical activities, attuned to individual needs, routines and goals, embedded in their own environment focusing on self-management to guarantee a more active lifestyle without the external coaching. Ideally a multidisciplinary team should monitor the patient yearly to guarantee the start of intervention when needed. However, infancy is the most important so-called sensitive period for later developmental outcome. We advocate that especially young infants will profit from early intervention, focusing on skill acquisition and strength training to increase motor development.[76,77,95,96]

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Appendix A

Search strings

Psychomotor development, motor performance, and fitness in PWS	
PubMed	(((((((Psychomotor performance[MeSH Terms]) OR physical fitness[MeSH Terms]) OR motor[Title/Abstract]) OR psychomotor[Title/Abstract]) OR fitness[Title/Abstract])) AND (((Prader Willi syndrome[MeSH Terms])) OR ((("Prader"[Title/Abstract]) AND "Willi"[Title/Abstract]) AND "syndrome"[Title/Abstract]))) AND (English[lang] AND ("1980/1/1"[PDAT] : "2011/6/30"[PDAT]))
CINAHL	((AB "Psychomotor") or (AB "motor") or (AB fitness)) and (TI Prader and TI willi and TI syndrome) Limiters - Published Date from: 198001-201106; Language: English Search modes - Boolean/Phrase
PsycINFO	(Psychomotor or motor or fitness).ab. and (prader and willi and syndrome).ti. limit 24 to (english language and yr="1980 - 2011")
Embase	(Psychomotor or motor or fitness).ab. and (prader and willi and syndrome).ti. limit 24 to (english language and yr="1980 - 2011")
Physical training or physical therapy in PWS	
PubMed	((((Physical therapy modalities[MeSH Terms])) OR (("motor"[Title/Abstract]) AND "training"[Title/Abstract])) OR ((((("physical"[Title/Abstract]) AND "training"[Title/Abstract])) OR ("physical therapy"[Title/Abstract]))) AND (((Prader Willi syndrome[MeSH Terms])) OR ((("Prader"[Title/Abstract]) AND "Willi"[Title/Abstract]) AND "syndrome"[Title/Abstract])) AND (English[lang] AND ("1980/1/1"[PDAT]: "2011/6/30"[PDAT]))
CINAHL	((AB "motor training") or (AB "physical training") or (AB "physical therapy")) and (TI Prader and TI willi and TI syndrome) Limiters - Published Date from: 198001-201106; Language: English Search modes - Boolean/Phrase
PsycINFO	(motor training or physical training or physical therapy).ab. and (prader and willi and syndrome).ti. limit 24 to (english language and yr="1980 - 2011")
Embase	(motor training or physical training or physical therapy).ab. and (prader and willi and syndrome).ti. limit 24 to (english language and yr="1980 - 2011")

4

Growth hormone combined with child-specific motor training improves motor development in infants with Prader-Willi syndrome: A randomized controlled trial

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Abstract

Although severe motor problems in infants with Prader-Willi syndrome (PWS) are striking, motor development has never been studied longitudinally and the results of growth hormone (GH) treatment on motor development are contradictory. The authors studied whether GH treatment can enhance the effect of physical training on motor development in infants with PWS. Twenty-two infants were followed for two years during a randomized controlled trial. The treatment and control groups began GH treatment after baseline or following a control period, respectively. Both groups followed a child-specific physical training program. Motor performance was measured every three months. Multilevel regression analysis revealed that motor development differed significantly between infants ($p < .001$), and this could be partially explained by baseline motor developmental level ($p < .01$). GH treatment enhanced the effects of child-specific physical training on both motor developmental rate and motor developmental potential. Moreover, this effect was more pronounced when GH treatment was initiated at a younger age.

Introduction

Prader-Willi syndrome (PWS) results from a lack of expression of the paternally derived chromosome 15q11–q13 resulting from a deletion,[1] uniparental disomy,[2] imprinting center defects or balanced translocations[3]. The estimated prevalence of PWS is one in 10,000–30,000 live births.[4] The most significant features of PWS include hypogonadism, hyperphagia, obesity, short stature, mild dysmorphic facial features, and cognitive and behavioral deficits.[2,5] Cognitive defects include mental retardation and problems with attention, short term memory, auditory processing, and sequential processing.[6-8] Social functioning and the ability to interpret social information is poor, even considering their decreased intellectual abilities.[9] The emergence of maladaptive behaviors such as hyperphagia, temper tantrums, mood fluctuations, obsessive and compulsive behaviors, ritualistic, repetitive and perseverative behaviors, and skin-picking, appears to escalate with age, increasing in severity and intensity.[6,10,11] In infancy, feeding difficulties, failure to thrive, and severe muscular hypotonia and weakness are most prominent, resulting into serious developmental delay.[5,12-14] Pediatric physical therapy and growth hormone (GH) treatment are prescribed to improve psychomotor development in these patients.[5,15] We therefore hypothesized that GH treatment could improve the effect of specialized physical training on motor development in PWS infants.

Motor activity is a vital factor during early development, as each new motor skill creates new opportunities for perceptual, motor, and social exploration, allowing the infant to interact with and learn from its environment.[16] In the first few years of life, brain development is guided by a continuous interaction between the organism and its environment,[17-20] and motor activity is a key factor in this interaction.[16,21] PWS infants have major difficulties overcoming gravitational forces during activities such as stabilizing and positioning the head, reaching, grasping, rolling over, and sitting or standing upright. In typical development 90% of infants is able to stabilize and position the head at about 3.5 months of age, sitting at 8 months, crawling at 9 months, and walking at 13.5 months.[22] In PWS infants, head control usually does not occur until eight months of age, sitting at 12 months, crawling at 19 months, and walking at 27–32 months.[23,24] For brain development of PWS infants, it is important to stimulate motor development early in life.

Motor problems in PWS patients are presumed to be related to an abnormal high fat-muscle ratio. In normally developing infants, this ratio is important for overcoming gravitational forces and executing the stepping reflex.[25] In PWS patients, however, from birth onwards, fat mass is increased and muscle mass is decreased.[26-28] Thus, the body fat level of PWS infants ranges from 28 to 32%, whereas 24% is normal;[26-28] in childhood, fat mass increases to 36–55%,[29-31] whereas 18% is normal. Finally, muscle mass is decreased by 25–37% in PWS patients compared to normal individuals.[32]

In addition, some indications for abnormal muscle fiber type distribution,[33] altered mitochondrial function,[34] and hypo-excitability of motor cortical areas[35] are found. Because PWS patients have decreased motor activity[36,37] – even before birth[38] – these muscle abnormalities could be part of a vicious circle in which decreased muscle strength and hypotonia lead to an inability to

overcome gravity, which can hamper skill learning and motor activity, which in itself contributes to muscle abnormalities.

Physical training positively affects body composition, muscle force, and skill acquisition in PWS patients.[39] Therefore, pediatricians recommend pediatric physical therapy for infants as part of a multidisciplinary approach to manage this complex disorder.[5,15] Moreover, parents require coaching for how to care for their extremely hypotonic infant. However, studies that focus on the principles of such a program are lacking. We believe that intervention should focus on manipulating the learning environment in such a way that the child can overcome gravity, improve muscle strength, and learn motor skills by repeated practice.

GH treatment not only normalizes height,[40] it also reduces the fat-muscle ratio in children and infants with PWS.[27,29,40,41] A recent review on the effect of GH and training on motor performance in Prader Willi Syndrome pointed out that in some studies, beneficial effects of GH on physical agility in older children have been reported.[29,39,42,43] Whether GH treatment also has a positive effect on motor development and motor performance in infants is unclear, because of contradictory results.[13,14,27,28,39,44] This could be due to small cohort sizes combined with high inter- and intra-individual variability of motor development among PWS infants.[14,28] There are some rare side effects of human GH therapy and because of concerns about unexpected death in PWS patients on account of respiratory obstruction, sleep polysomnography and assessment for enlarged tonsils and adenoids are recommended during GH treatment.[5]

The objective of this study was to test the hypothesis that GH treatment can enhance the effect of child-specific physical training on motor development in PWS infants.

Methods

Design

In this two-year longitudinal single-blinded controlled trial, after stratification for age, PWS infants were randomized [1:1] to either the GH group, which received Genotropin at 1 mg/m^2/day, or to a control group, which received GH following a six-month control period. Both groups received intense standardized individually graded physical training. For each child nine measurements were collected at three-month intervals for two years. The study leader (M.N.), researcher (L.R.), and the two well-trained pediatric physical therapists (A.Z. and I.D.) were blinded for randomization.

Patients

All parents of newly diagnosed PWS patients in the Netherlands were approached by the Dutch Growth Research Foundation (DGRF) via their pediatrician. The DGRF registered the infants and invited the parents to participate in the Dutch PWS GH study (ISRCTN49726762). From September 2006 to June 2010, parents of infants up to the age of 36 months were also asked to participate in the pediatric physical therapy study at Radboud university medical center (Radboudumc). After receiving written informed consent from the parents, the patients were

randomized by the DGRF, using a computer-generated list of random numbers. This study was approved by the Medical Ethics Committees of Erasmus Medical Centre Rotterdam and Radboudumc.

Physical training

The Radboudumc Pediatric Physical Therapy Department developed an individually tailored at home intervention program for PWS infants in which the learning environment can be manipulated in such a way that the child can overcome gravity, increase muscle strength, and learn motor skills by repeated practice. Parents and involved pediatric physical therapists were coached to care for and simultaneously stimulate the motor development of PWS infants by two pediatric physical therapists from the center (A.Z. and I.D.) conform the principles defined in the protocol and based on the findings in the diagnostic session (Box 1). Because this was a newly developed treatment intervention strategies and dilemmas were discussed in monthly meetings between the study leader (M.N.), the researcher (L.R.) and the pediatric physical therapists (A.Z. and I.D.). So goal setting and intervention strategy were adapted to the individual developmental stage of the infant and using progressive load on muscle force. Parents and involved physical therapists were not blinded for randomization of GH, but were instructed not to inform the physical therapists and researcher in the Radboudumc.

At baseline and every three months thereafter (with alternating hospital and home visits), questions from parents and therapists were discussed, motor performance was evaluated by the researcher and pediatric physical therapist at Radboudumc, and training goals and physical exercises were discussed (Box 1). The program was attuned to the motor level, needs of the parents and therapists, and interests of the child and consisted of handling instructions and muscle strength and skill acquisition exercises. Parents and pediatric physical therapists were instructed by oral and written reports to integrate the exercises into daily care to increase compliance and to contact the center by phone or email.

Outcome measures at baseline and every three months

Growth was measured using weight and supine length or height, as appropriate. *Motor performance* was assessed using three standardized motor development tests: the Alberta Infant Motor Scale (AIMS); the Motor Scale of the Bayley Scales of Infant Development, 2nd edition (BSID-II); and the Gross Motor Function Measure (GMFM). Each test focuses on a different motor development domain. All three motor developmental tests are valid and reliable.[22,45-50] Assessments were performed by two well-trained pediatric physiotherapists (A.Z. and I.D.).

AIMS is a norm-referenced instrument focusing on the gross motor milestones (e.g. rolling, standing, and walking), as well as the components needed to attain these milestones, such as the infants posture, weight-bearing, and anti-gravity movements. It assesses motor development in four positions: prone, supine, sitting, and standing, from 0–18 months of age.[48] Raw scores and

developmental age were used in accordance with the test instructions. Developmental percentage was calculated as follows: [(developmental age)/(chronological age) × 100].[14]

Box 1: The individually tailored at home pediatric physical therapy program

Evaluation of motor performance (at three–month intervals for two years)
- **Structured interview**: the problems encountered when caring for the child, health problems, the progress of general and motor development, and specific questions from the parents and/or therapists were discussed.
- **Diagnostic observation**: Motor performance, motor problems based on muscle force limitations, and problems in handling the infant were observed.
- **Motor development tests**: Three motor development tests were performed (see the Methods section Assessment of motor performance).
- **Detection of motor development potential**: Motor performance was playfully manipulated and stimulated to test which strategies the child needed to achieve the next step in motor development. Moreover, handling and support/backing materials were offered and tested.
- **Demonstration of physical exercises**: Specific exercises for muscle groups at 70% of maximum muscle strength level related to the aimed developmental skills (next step) were conducted and demonstrated to the parents; moreover, playful skill training appropriate to the child's motor, cognitive and social emotional levels (e.g., sitting and reaching) was offered.
- **Final conclusion**: Motor development progress, results of the diagnostic observation, the training goals, exercises and advice to support the infant were discussed.

Report (at three-month intervals for two years)
- **Interview**: summary of the interview
- **Motor performance**: findings of the diagnostic observation
- **Test results**: results of the motor development tests
- **Training goals**: focus and goals of training
- **Support and backing**: materials were offered or the parents were told how these materials could be obtained
- **Exercises**: exercises were embedded in the daily activities and were: 1) functionally related to the comprehension of particular motor milestones, 2) focused on the manipulation of the learning environment in such a way that both muscle strength training (8-10 repetitions at 70% of maximum force) and motor skill training (easy repetitions as long as possible) were combined, and 3) related to the specific needs of the child and parents

Communication and exchange (throughout the entire program)
- The report was provided to the parents and the local therapist.
- At the beginning and throughout the program, the local therapist was contacted to discuss the training program.
- If specific problems appeared during the program, they were discussed with the parents and the local therapist.
- Both the parents and the local therapist could contact the expertise center via telephone or email.

The motor scale of the BSID-II is a norm-referenced instrument to evaluate gross and fine motor skills from 1–42 months of age.[45] For example, a gross motor item is climbing the stairs with alternating legs and a fine motor item is threading three beads. From the raw scores, developmental age equivalents were determined in accordance with the test instructions. The raw score, developmental age, and developmental percentage were used as outcome measures.
GMFM was originally developed and validated to assess motor function in children with cerebral palsy[50] and contains 88 items that are grouped into five dimensions: (1) lying and rolling, (2) sitting, (3) crawling and kneeling, (4) standing, and (5) walking, running, and jumping. Typically developing children can perform all 88 items by 5 years of age. The items are scored on a 4-point ordinal scale (0 = does not initiate, 1 = initiates <10% of activity, 2 = partially completes 10% to

<100% of activity, 3 = completes activity), which makes it possible to also score items the child does not yet completely accomplish. The total GMFM score was used as an outcome measure by averaging the percentage scores of the five dimensions.

Statistical analyses

Descriptive statistics were used to characterize and compare the groups at baseline. To evaluate whether the addition of GH treatment to child-specific physical training improved motor development over time, taking individual motor development patterns into account, multilevel regression analysis (MLRA) was used. This technique is well-suited to analyze data related to developmental processes,[51] as it accounts for both within-patient variations over time (level one) and between-patient variability (level two). For each motor development test, the same strategy was used to develop a regression model that could adequately predict the observed individual motor development patterns using age, baseline motor developmental level (BSID-II developmental percentage at baseline), and GH as explaining variables. Pearson's correlations were calculated between the age when the intervention had started and (1) the age at which the infant walked independently, (2) the age at which the end of the AIMS was reached, (3) the BSID-II end score, and (4) the GMFM end score. To evaluate the predictive power of the models, we used a fit measure (see Appendix). MLRA was performed using "Imer" in the software package R; other statistical analyses were performed using SPSS 18.0.

Results

Participants

From September 2006 through June 2010, 27 infants were registered at the DGRF. Two parents chose not to participate in the study due to long travel distance, and three infants were excluded (one was older than 36 months, one had previous neurological trauma, and one moved out of the country); parents of two infants refused to start GH treatment at such a young age, but wanted to participate in the training, these infants were not randomized but added to the control group. This allowed to sample data over a longer period in the control condition. Hence, 20 infants were randomized, with 10 and 10 (+2) infants in the GH and control groups, respectively. In the control group two had a control period of 24 months, two of 12 months, and eight of six months (Figure 1). Originally we planned a control period of 12 months, however, after the start of the study the results of Festen et al.[14] pointed into the direction of effectiveness of GH. So based on ethical considerations we shortened the waiting period in the control group to six months and used a statistical analyzing method suitable to cope with differences in control periods. The training program began at a mean age of 12.9 months (SD = 7.1, range: 4.7–31.8), and the patients received GH for the first time at a mean age of 17.5 months (SD = 7.3, range: 6.7–34.2). The clinical characteristics of the patients, their ethnicity, and genetic subtypes are presented in Table 1.

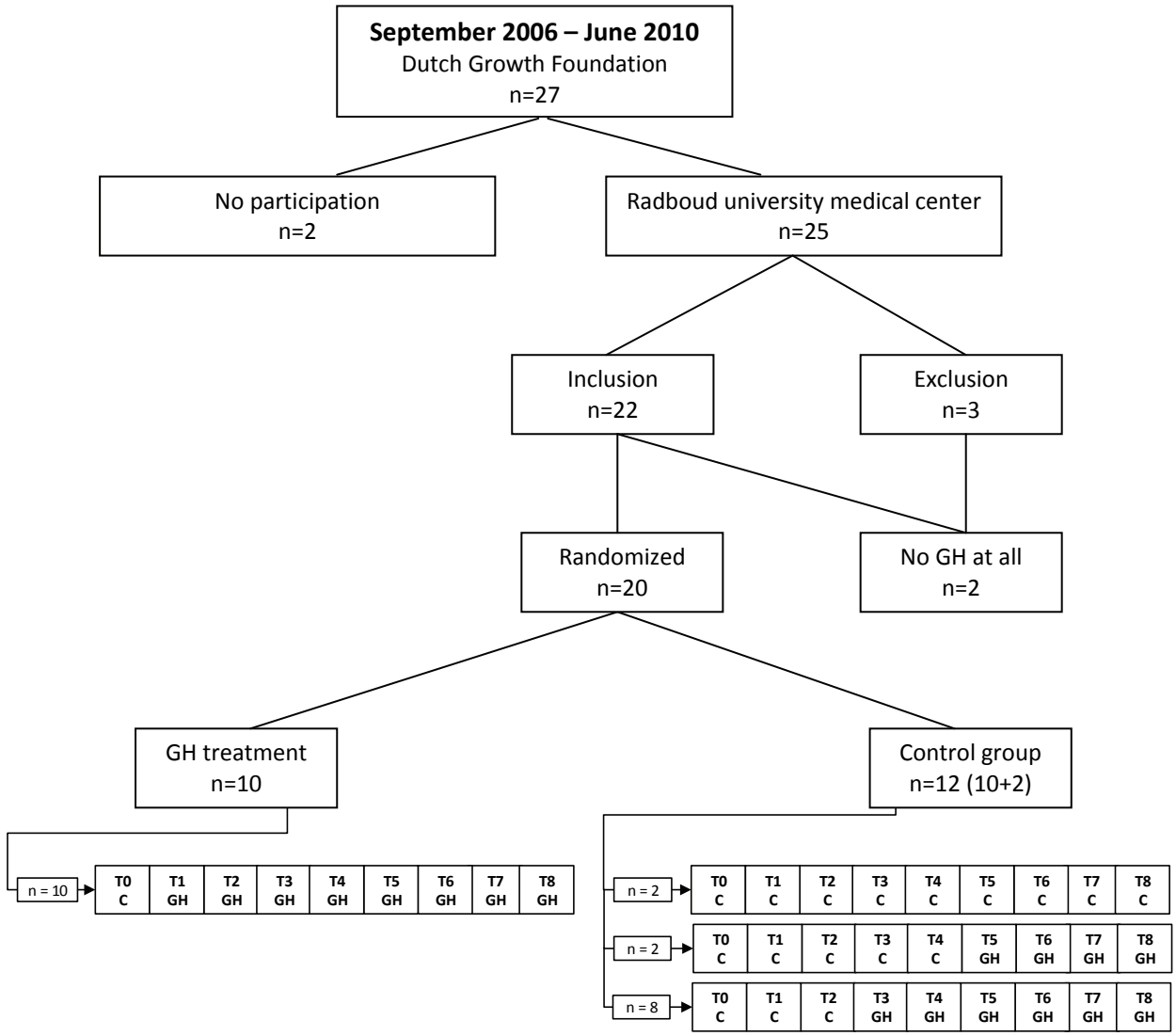

Figure 1. Flowchart subject selection, inclusion and distribution among two groups
c = a control observation in which the infant did not receive GH treatment,
GH = a observation in which the infant did receive GH treatment).

Effects of GH treatment on motor development

A one-way ANOVA revealed no difference between the control and GH groups at baseline (Table 1). Moreover, age, height, weight, AIMS and BSID-II developmental percentage were similar among the PWS infants with various genotypes. From all 22 patients, four observations were missing, and in five infants, an extra measurement before inclusion in the GH study was performed; thus, a total of 199 measurements were analyzed. The observed longitudinal data of the PWS infants from the motor development tests are shown in Figure 2a–c.

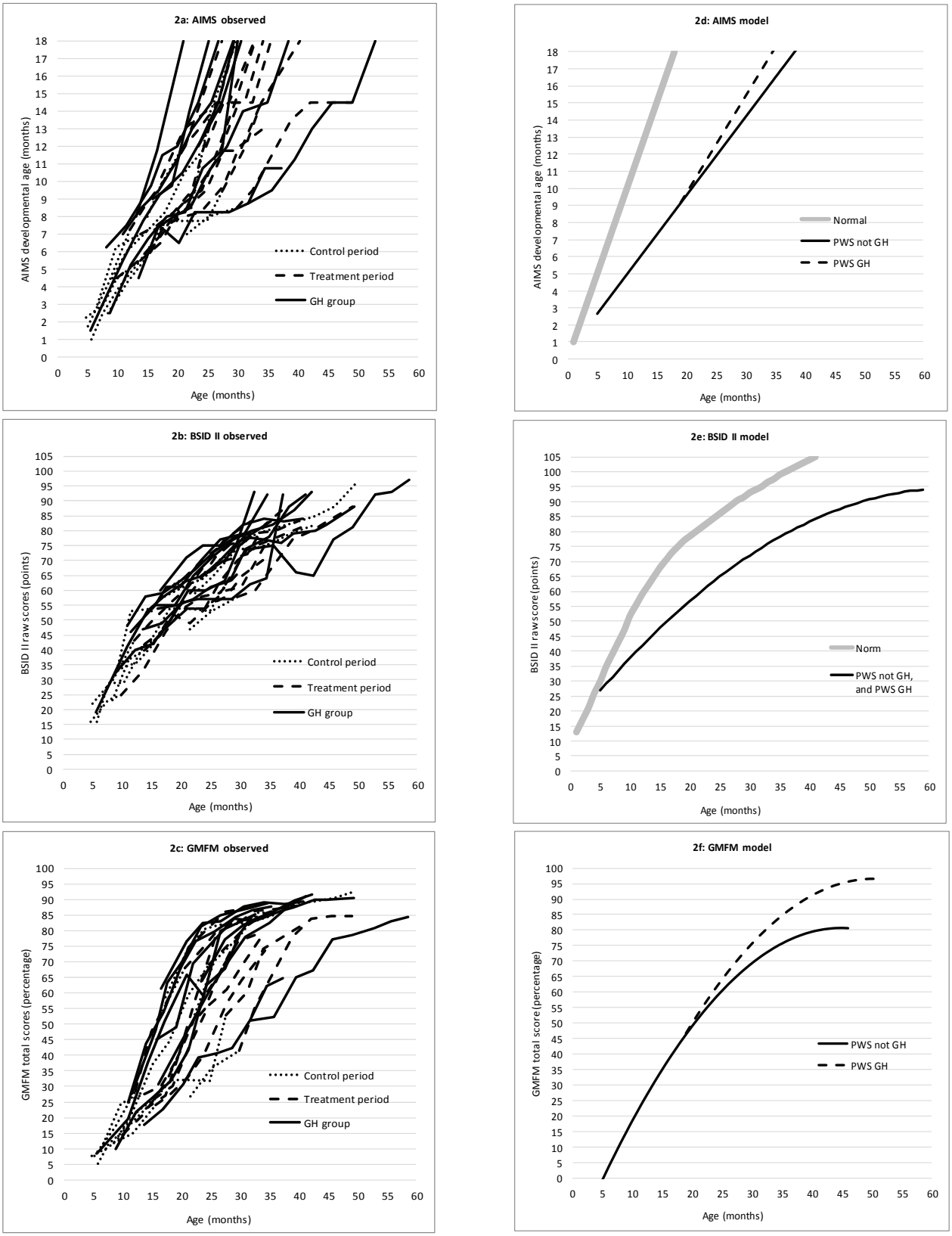

Figure 2. The observed longitudinal data (panels a-c) with their corresponding regression models (panels d-f)
In panel a-c each line represents an individual patient.
In the panels d and e the norm reference values are also presented.

Individual motor development patterns were discerned in all three tests. With respect to the GMFM, infants reached their maximum potential during the study; above a score of 79, motor development plateaued before the maximum total GMFM score of 100 could be reached (Figure 2c).

Table 1. Clinical characteristics at baseline for all patients, the control group, and the GH-treated group

N	All Patients 22 Mean (SD)	Control group 12 Mean (SD)	GH group 10 Mean (SD)	Control group vs. GH group p-value
Gender (M/F)	14/8	9/3	5/5	.44
Ethnicity (Dutch/non-Dutch[a])	19/3	10/2	9/1	.57
Genetic subtype (Deletion/UPD[b]/unknown)	10/9/3	5/4/3	5/5/0	1.0
Age, in months	12.9 (7.1)	11.7 (6.3)	14.2 (8.1)	.43
Height, in SDS	-1.8 (1.2)	-2.0 (1.1)	-1.6 (1.2)	.40
Weight, in SDS	-1.3 (1.5)	-1.6 (1.5)	-0.9 (1.6)	.29
AIMS raw score	24.8 (14.7)	21.5 (14.3)	28.8 (15.0)	.26
AIMS developmental age, in months	5.6 (3.2)	4.9 (3.0)	6.5 (3.3)	.26
AIMS developmental %[c]	43.7 (14.8)	40.9 (11.4)	46.9 (18.1)	.36
GMFM total score	26.8 (18.9)	23.7 (18.8)	30.4 (19.3)	.43
BSID-II raw score	40.6 (17.1)	35.8 (15.9)	46.5 (17.5)	.15
BSID-II developmental age, in months	7.3 (4.3)	6.0 (3.7)	8.9 (4.6)	.13
BSID-II developmental %[c]	55.0 (15.0)	49.5 (13.4)	61.6 (14.7)	.06

[a] All infants were born in the Netherlands, but three families came from the Middle East.
[b] UPD = uniparental maternal disomy
[c] developmental % = [(developmental age/chronological age) × 100]

In the BSID-II, motor developmental rate also decreased over time, albeit more gradually, and none of the infants reached the test's ceiling (Fig. 2b). In the AIMS, the increase in motor development was more linear, although the test's ceiling was reached at a much older age than normal (Figure 2a). All 199 observations – 62 of which were control observations –were included in the MLRA regression model of the GMFM and BSID-II. The control observations include the baseline measurements of both groups and the control measurements taken during the control period of the infants in the control group. In the AIMS model, all 158 observations – of which 58 were control observations – were included before the test's ceiling value was reached.

Motor development measured by the AIMS (gross motor function)

Motor developmental age was used in the AIMS regression model (Figure 2d and Table 2). In the empty model, 95% of the original AIMS values were at a maximum distance of 8.47 developmental months from the mean AIMS value [1.96 × 4.32]. In the final model, using age, developmental level at baseline, and GH as explanatory variables, the maximum distance was 1.90 developmental months [1.96 × 0.97]. Therefore, the model reduced the 95% interval of unexplained AIMS variation by 78% [1–(1.90/8.47)]. In this model, motor development was expressed by a random linear component, age (mean: 0.46; SD: 0.17; $p < 001$). Motor development varied significantly between patients, and the model predicted that a PWS infant

with an average motor development rate needs 2.2 months [1/0.46] to improve one month in motor developmental age on the AIMS; with a high rate of motor development, this improvement takes 1.6 months, and with a low rate of development, improvement takes 3.4 months. In accordance with the model, the age at which a PWS infant can walk independently and reach the end of the test are presented in Table 3.

Table 2. Results of the three multi-level regression models, including the explaining variables (age, GH, and baseline level) and the interactions with their factor

	AIMS	BSID-II	GMFM
Intercept	0.33 (1.34)	14.84 (9.31)	-22.29 (4.36)
Age	0.46 (0.17) ***	2.49 (1.04)***	4.58 (1.27)***
Age^2	-	-0.02 (0.02)**	-0.05 (0.03)***
GH	0.61*	-0.27	3.03*
Baseline-level-c	0.05***	0.28 ***	0.50**
GH × age-c	0.11***	-	0.55***
Baseline-level-c × age-c	-	-	-0.01**
Baseline-level-c × $age\text{-}c^2$	-	-	-0.003***
SD residual	0.97	3.70	3.91
SD empty model	4.32	17.77	25.90
Reduction unexplained variation[a]	78%	79%	85%

**p <.05, **p <.01, ***p <.001 two-sided*

Values in parentheses represent SD.

[a]The empty model and the final model were compared and used as a measure of the model fit (see Appendix).

Explaining variables: Age = age in months (age- expresses linear developmental rate); Age^2 = age squared (age^2- expresses decrease in motor developmental rate over time); Age-c = age-centered; $Age\text{-}c^2$ = age-centered and squared; GH = a Boolean variable (0 = no, 1 = yes); Baseline-level-c = BSID-II developmental percentage at baseline centered.

Baseline motor developmental level had a significant effect on motor development over time (0.05; $p < 001$). Infants with a high initial motor developmental level (70%) performed consistently better over time than infants with a lower developmental level (40%), with a difference of 1.5 months in developmental age.

Age and GH had significant interaction effects on the AIMS (0.11; $p < 001$); approximately six months after starting treatment (i.e., at the age of 24 months), the effect of GH was both positive and significant (0.61; $p < 05$). GH increased the developmental rate of PWS infants (Figure 2d and Table 3), regardless of their initial motor developmental rate.

Motor development measured by the BSID-II (gross and fine motor function)

In the BSID-II regression model, the raw BSID-II scores were used (Figure 2e and Table 2). In the final model, using age, developmental level at baseline, and GH as explanatory variables, the unexplained BSID-II variance was reduced by 79% compared to the empty model. In this model, motor development was expressed as a combination of a linear and quadratic age component; the quadratic component represented the gradual decrease in motor development rate over

time (Figure 2b). The intercept and age components were random, indicating that motor development varied significantly between patients (mean intercept = 16.89; SD = 10.60; mean age = 2.36; SD = 1.16; $p < 001$; mean age^2 = −0.02; SD = 0.02; $p < 001$). The model predicts that all infants will reach the same level at approximately the same age, as over time, the decrease in motor development is faster in rapidly developing infants, gradual in infants who develop at an average rate, and slower in slowly developing infants. In accordance with the model, the ages at which a PWS infant can walk independently and reach a maximal BSID II score of 82, are presented in Table 3.

The BSID-II also revealed a significant effect of baseline motor developmental level (0.28; $p < 001$). The model predicts that infants with a high level of motor development at baseline will perform consistently better over time than infants with a lower developmental level. A PWS infant with an average developmental rate and an initial motor developmental level of 70% can walk independently at 24.3 months compared with 31.3 months for an infant with a motor level of 40%. Unlike the AIMS, the BSID-II detected no significant effect of GH on motor development.

Table 3. The average age at which PWS infants could walk and the prediction of the models for the age at which PWS infants could walk and reach the end or maximum test scores

	Observed Walking mean (SD)	AIMS Walking (dev. age[a] 13) mean	BSID-II Walking (RS[b] 68) mean	GMFM Walking (score 70) mean	AIMS End test (dev. age[a] 18) Mean	BSID-II Max.[c] (RS[b] 82) mean	GMFM Max.[c] (score 79) mean
Mean	27.0 (5.8)	27.5	27.1	30.5	38.3	41.9	39.4
Fast	21.2	22.3	24.0	24.6	30.2	40.2	30.5
Slow	32.8	38.6	30.4	35.7	55.8	42.3	41.9
GH	-	25.7	-	27.5	34.5	-	31.7

[a] dev. age = developmental age in months.
[b] RS = raw score.
[c] Max. = maximal score just before the decrease in motor developmental rate.

Motor development measured by the GMFM (fundamental gross motor skills)

In the GMFM regression model, the total GMFM score was used (Figure 2f and Table 2). In the final model, using all three explanatory variables, the unexplained GMFM variance was reduced by 85%. In this model, motor development was expressed as a combination of a linear and quadratic age component. The quadratic component represented the abrupt drop in motor development rate above a score of 79 (Figure 2c). The intercept and age components were random, indicating that motor development varied among patients (mean intercept = −24.05; SD = 0.0002; mean age = 4.78; SD = 0.90; $p < 001$; mean age^2 = −0.06; SD = 0.02, $p < 001$). In accordance with the model, the ages at which the PWS infants could walk independently and reach a maximal GMFM score of 79 are presented in Table 3.

A significant effect of baseline motor developmental level (0.50; $p < 01$) and a positive interaction between baseline motor developmental level and age were found (baseline-level × age: −0.01; $p < 001$; baseline-level × age^2: −0.003; $p < 001$). A low initial level of development resulted in a

slower developmental rate; however, because motor developmental rate decreases above a score of 79, the model predicts that over time all infants will reach approximately the same maximal score regardless of their initial baseline level.

There was a significant interaction effect of age and GH (0.55; $p < 001$); approximately six months after starting treatment at the age of 24 months, this effect was positive and significant (3.03; $p < 05$). The model predicts that motor developmental rate increases following GH treatment; the child's maximum potential increased by 16 points, from 70 to 95 (Figure 2f). In PWS infants, 79 points indicates a child who can walk independently and stand on one leg for a few seconds, but is unable to run and has a head lag when pulling to a sitting position. A score of 95 points indicates a PWS infant who can run, step over a stick at knee-height, and pull to a sitting position without head lag, but is unable to jump or climb stairs using an alternating step gait.

Correlations between the start of treatment and motor development

Twenty PWS infants were ultimately able to walk independently during the study (mean: 27 months, SD: 5.8, range: 17.5–42.4); 19 of these infants were treated with GH. The infants who received GH at a younger age walked earlier than those who started at an older age ($p < .05$, $r = 0.56$). Fifteen of the 20 infants who received GH during the study reached the end of the AIMS (the remaining five infants were not yet able to squat and stand up). The infants who received GH at a younger age reached the end of the AIMS earlier than those who started at an older age ($p < .01$, $r = 0.71$). The score at which the GMFM developmental rate began to decrease and the age at which this score was reached varied among the patients. Therefore, for each child, the highest GMFM score attained before developmental rate begun to decrease was divided by that child's age. This age-corrected measure correlated significantly with the age at which GH was started ($p < .05$, $r = 0.52$). The infants who started GH at a younger age had a higher maximum GMFM score than those who started at an older age. There was no correlation between the age at which GH was started and motor development measured using the BSID-II.

Discussion

Although motor development can vary widely among infants with PWS, these patients have a specific pattern of development. In this study, the infants with PWS took approximately twice as long to reach motor development milestones than infants who develop typically (Figure 2d); in addition, over time, motor developmental rate decreased and appeared to plateau in PWS infants (Figure 2b and c). The results support our hypothesis that GH enhances the effect of child-specific physical training on motor development. The combination of GH and training resulted in a significant clinically relevant improvement in motor development (Figure 2d and f). Despite this positive effect, a large delay in motor development remained. Furthermore, we found indications that early treatment with GH results in an earlier increase in motor developmental rate and a higher final level of development.

Consistent with Festen et al.,[14] but in contrast to the findings of Eiholzer et al.,[13] we found a positive effect of GH on motor development. In the study of Eiholzer et al.[13] a comparable group of infants participated. The control group received CoQ10 supplementation, the experimental group GH. The authors concluded that the similar improvement in motor development in both groups presumably reflect an age related phenomenon related to the early diagnosis and the addition of appropriate care independent of GH or CoQ10 supplementation. The fact that we did demonstrate an effect of GH on motor development is presumably the result of our more sophisticated study design, earlier intervention, and our standardized training program. Moreover we evaluated longitudinally over two years, assessing motor performance every three months, which made it possible to take individual differences in motor development between infants over time into account while evaluating an effect of GH using MLRA.

Some authors have suggested that GH is only effective when treatment is started at a young age and have reported that these infants could walk at an earlier age than typical PWS infants.[27,44] Because the effect of GH both increased over time and became significant later in development, the mean age at which the PWS infants in our study walked independently was similar to the age typically reported for PWS.[23,24,44,52] However, because we found a positive correlation between the age at which GH treatment began and the age at which the infants could walk, we estimate that had GH been given at a younger age, the infants would likely have walked even younger than 27 months. In this study, the control period of six months precluded us from starting GH treatment at an earlier age.

We also found that initial motor performance level was correlated with the motor developmental rate and the age of walking, which might reflect the genetic variance of our subjects. Thus, any comparisons between relatively small study groups are likely hindered by the complexity and variability of such influencing factors. The AIMS and GMFM clearly revealed a significant and clinically relevant positive effect of GH on motor development; the child's maximum motor potential increased with GH treatment, thereby resulting in a clear functional improvement. A GH-treated infant can ultimately run (after which its motor development will plateau), whereas an untreated infant will only achieve the ability to walk. However, the BSID-II revealed no effect of GH on motor development. Both the AIMS and GMFM focus on gross motor function (in which both fat-muscle ratio and muscle strength are critical factors), whereas the BSID-II focuses on both gross and fine motor functions. Thus, a test that includes fine motor skills may be less sensitive at detecting the effects of GH. Nevertheless, Festen et al.[14] detected an effect of GH treatment using the BSID-II, presumably because in contrast to our study, their control period was twelve months; therefore, they likely detected a larger difference in motor development between groups.

Strengths, limitations and guidance for future research

This is the first study in which motor development in PWS infants was studied longitudinally, thereby establishing a clear pattern of motor development. We included 22 infants whit PWS,

which is almost the whole Dutch population diagnosed between September 2006 through June 2010. Research in patient groups with low incidence are difficult to perform especially in children where you need to control for developmental changes. We tried to reach stable models by increase of the repeated measurements. The high number of repeated measures allowed us to analyze the data using MLRA, which has the advantage over the traditionally used ANOVA to account for inter- and intra-individual differences. Although in MLRA the amount of cases on the second level (that is 22 infants) is usually higher, we were able to achieve convergence. Moreover, all three models predicted the clinical observations well, as unexplained variation was reduced by ≥78%, and the models accurately predicted the age at which the infants could walk independently. Another weakness in our study is the waiting list design and the differences in control period. However, we corrected this in the chosen statistical design, which increases the generalizibility of the results. We used a combination of both GH and training. The optimal design would have been a study containing four groups of PWS infants: no intervention, physiotherapy only, GH-treated infants only, and both therapy and GH; however, the rarity of PWS precluded such a study design, which would be feasible only through a multi-national collaboration. Moreover, it would have been unethical to withhold physical therapy from these patients and information regarding how to manage a special-needs child from the parents. It was apparent that the parents and local pediatric physical therapists appreciated the intervention and the coaching from the Radboudumc. The standardized approach reduced variability in the way that motor development was supported and stimulated, however, as in all therapeutic interventions, differences in adherence and therapist–client interaction were possible sources of variation. We designed this study to test the effect in normal practice situation, which make the results more valid. We do not expect a systematic variation. This is confirmed by the high explained variation in the model by known influencing variables (age, motor developmental level at start, and GH).

PWS infants in our study reached their maximum motor developmental level before they could jump or climb stairs using an alternating step pattern. This is a deficit in the study design. We cannot say anything about the maximum level, but based on the literature we expect lifelong motor performance problems.[39] Long-term longitudinal research is needed to determine if – and at what age – these final motor milestones are reached and to evaluate the long-term effects of early intervention of GH and training on development. Nevertheless, our results emphasize that a combination of both GH treatment and physical training has a clear positive effect on motor development, and we recommend the addition of GH treatment to a physical therapy program in which parents learn to stimulate their infant's motor development and are encouraged to perform daily specific muscle strengthening exercises. We also advise pediatricians to initiate this treatment regimen as early as possible for maximum benefit to the patient. However, it is not clear if GH needs to be used lifelong, this needs to be tested in future studies.

Concerning future studies we emphasize that in such small patient groups longitudinal designs with repeated measurements are preferable above short-term trials. Moreover, more studies are needed to test the interaction between motor, cognitive, and psychosocial development.

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Appendix

Model fit

Using MLRA, it is rather complex to present a measure for the model fit, for example, R square.[51] Therefore, we derived a fit measure as follows: in an empty regression model with no predictors and only the intercept, the predicted value in the model is equal to the mean of the dependent variable, whereas the residual standard deviation is equal to the original standard deviation of the dependent variable. Assuming a normal distribution of the residuals, 95% of all observations will lie at a maximum distance of 1.96 standard deviations from the "predicted" value (i.e., the mean value). After adding explanatory variables to the model, the residual standard deviation can then be used in the same way to derive the maximum distance from the model predicted value for 95% of the observations. Using the ratio of the 95% distance in the full model and the ratio in the empty model, and then subtracting this ratio from 1, provides an approximate evaluation of the predictive power of the final model in terms of the proportional reduction of the 95% distance to the predicted value. This measurement can also be used to compare the fit of our final models for each of the three motor development tests. In this derivation, we assumed that both the model residuals and the test values were distributed normally.

5

Objective evaluation of muscle strength in infants with hypotonia and muscle weakness

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Abstract

The clinical evaluation of an infant with motor delay, muscle weakness, and/or hypotonia would improve considerably if muscle strength could be measured objectively and normal reference values were available. The authors developed a method to measure muscle strength in infants and tested 81 typically developing infants, 6–36 months of age, and 17 infants with Prader–Willi Syndrome (PWS) aged 24 months. The inter-rater reliability of the measurement method was good (ICC = .84) and the convergent validity was confirmed by high Pearson's correlations between muscle strength, age, height, and weight (r = .79–.85). A multiple linear regression model was developed to predict muscle strength based on age, height, and weight, explaining 73% of the variance in muscle strength. In infants with PWS, muscle strength was significantly decreased. Pearson's correlations showed that infants with PWS in which muscle strength was more severely affected also had a larger motor developmental delay (r = .75).

Introduction

Making a diagnosis in a hypotonic infant within the first year of life is challenging for pediatricians and pediatric physiotherapists. Despite advances in genetic technology and brain imaging techniques, the value of the clinical assessment cannot be overemphasized and ought to be the first step toward a diagnosis.[1-4] The clinical assessment of 'floppy' infants focuses on differentiating between hypotonia of central (60–80%) or peripheral (15–30%) origin.[2,4] Regardless of the anatomical substrata and etiology, hypotonic infants show diminished resistance in the muscles to passive movement, a frog-like posture, excessive joint mobility, inability to maintain normal posture against gravity, and motor developmental delay.[4] If, despite these features, muscle strength is relatively normal then a central origin is most likely, whereas profound muscle weakness indicates a peripheral origin.[2-5] Evaluation of muscle strength in infants is difficult since no objective measurement methods are available. Clinicians observe the antigravity movements of the infant and test muscle strength manually using several maneuvers, such as the so-called 'pulling to sit', 'scarf sign', 'shoulder suspension', and 'ventral suspension'.[5,6] However, on the basis of these observations it is still difficult to determine whether the infant suffers from both hypotonia and muscle weakness or from hypotonia only.[5] Quantifying muscle strength in infants would enable us to determine to what extent muscle strength is diminished in relation to typically developing infants, and to evaluate effects of treatment on muscle strength.

Techniques to assess muscle strength in children and adults are well established and have been validated. Examples are manual muscle testing (MMT), hand-held dynamometer (HHD) and isokinetic dynamometry. MMT is used in accordance with the Medical Research Council scale, in which strength is classified on a 5-point scale.[7] When performed by very experienced assessors, the intra- and inter-rater reliability of MMT are satisfactory.[8,9] Unfortunately MMT has a limited sensitivity and specificity for detecting muscle weakness, particularly in patients with mild muscle weakness.[10] HHD has been validated[11,12] and its intra- and inter-rater reliability are very satisfactory for children,[8,13,14] even children as young as 30 months of age[15,16]. Isokinetic dynamometry is considered to be the golden standard. It is not used regularly for children, as the complex equipment used for adults requires extensive adaptations to fit to the various anthropometric characteristics of children.[17] Unfortunately, none of these methods are suitable, as infants are not able to generate maximum muscle strength in a muscle group on demand.[14-16] Therefore, we developed a new measurement method to quantify muscle strength in infants and toddlers, the so-called "Infant Muscle Strength meter" (IMS-meter). In infants aged six months and older, it is natural to pull a desirable object. Based on this behavior, we developed a pulling task to evoke a maximal pulling action and we then determined whether maximum muscle strength during such a spontaneous reaction in typically developing infants and toddlers between six and 36 months of age can be measured reliably. Because another measurement method to measure muscle strength in infants is lacking, it is not possible to compare the IMS-meter to another muscle strength test. Therefore, the convergent validity was evaluated by testing its correlation with age, height, and weight, because muscle strength is highly related to these

factors.[16,18,19] A model was developed based on sampled data in a convenient cohort of typically developing infants. With this model a prediction for typical muscle strength can be made, and this prediction can be compared to muscle strength in hypotonic infants. As an example to show how the IMS-meter can reveal understanding in the extent to which muscle strength is affected in hypotonic infants, we tested 24 months old infants with Prader–Willi syndrome (PWS), in which hypotonia, muscle weakness, and seriously delayed motor development are characteristics.[20,21]

Methods

Participants

Typically developing infants, divided in seven age groups of 6, 9, 12, 18, 24, 30, and 36 months (age range ± 21 days), were recruited in 2010 through an advertisement in a regional newspaper and an information leaflet in Bernhoven Hospital (general hospital) in Veghel, The Netherlands. Information about the child's health status was obtained from the parents during a telephone contact. If the infant was born at term, healthy, and typically developing, an appointment was scheduled. All parents gave written informed consent and the study was approved by the Medical Ethical Committee. All participating infants with PWS visited the department of pediatric physical therapy at the Radboud University Medical Center regularly. For this study we used muscle strength data measured with the IMS-meter at the age of 24 months. All parents gave written informed consent and the study was approved by the Medical Ethical Committee.

Measurements

For all children, height in centimeters and weight in grams were measured using a calibrated length meter and weight scale. Motor performance was assessed with the Motor Scale of the Bayley Scales of Infant Development, 2nd edition (BSID-II). This is a norm-referenced test to assess gross and fine motor skills between the age of one and 42 months.[22,23] In the typically developing infants, from the raw scores a psychomotor developmental index (PDI) was obtained to verify if motor development was within the normal range (PDI mean (100) ± 1 SD (15)). Because of severe motor developmental delay in infants with PWS, PDI-scores are all below -2 SD; [24] therefore, we calculated a developmental percentage by calculating, conform the manual, the developmental age and dividing this by chronological age multiplied by 100.[24] Afterwards the infant muscle strength (IMS) was measured.

To establish inter-rater reliability of the IMS-meter, IMS was measured two times by two different assessors in a convenient subgroup of 50% of the typically developing infants. The assessors were blinded to each other's scores, and whether assessor 1 (L.R.) or assessor 2 (M.L. or R.K.) performed the pulling task the first or second time, was randomized.

Infant muscle strength measurement

The IMS-meter contained a children's chair (Jenx corner seat) attached to a metal platform with a strength sensor, a laptop installed with Das Wizard software (version 3.00), and a Matlab program (R2006b) to analyze the output data. The strength sensor was sensitive to 250 Newton (Newport/Omega model LCEB-50) and had an error margin of .1%. During the study, the IMS-meter was calibrated regularly. The IMS-meter provided accurate and consistent measurements with a measurement error <1% and a coefficient of variance of 7.1%. Data were sampled with a frequency of 100 Hz and stored in an Excel data file.

The infant was positioned in the chair, which was adapted to anthropometric characteristics of the child so that the trunk, shoulders, and hip were fixed and the infant's feet could not touch the floor (Figure 1).

Figure 1. Muscle strength measurement in a 18 month old boy using the IMS-meter
Photograph by Thomas de Wit.

As pulling strength depends on the joint angle positions, the arms of the child were placed on a table at chest height and the toy was presented straight in front of the child above the table during the pulling actions. During the task, the child spontaneously used optimal comfortable arm joint position for pulling while the tester preserved that pulling was performed just above the table without touching it with the arms. The assessor presented a desirable attractive toy from stiff material to the child, which was easy for children aged 6–36 months old to grasp with either one or both hands (Figure 1). When the child's attention was caught, a kind of game began

in which the child tried to obtain the toy from the assessor. When the child pulled the toy the assessor gave counter strength to evoke maximum pulling strength. The assessor tried to obtain pulling actions of two seconds or more to be sure that the maximum strength was reached. During the task at least three pulling actions were performed in which the infant was encouraged to pull maximally. More pulling actions were carried out if the assessor felt the child's effort was incomplete. The pulling action with the highest peak value was used as outcome for IMS. To differentiate in the analysis phase between strength output generated by pulling activities and strength output generated by other movements of the child, the assessor used an electronic marker whenever the child was pulling and all measurements were recorded on film. Pulling data were stored in an excel file, which was read out by a Matlab analysis program that visually presented the pulling curves. In a pilot study pulling curves were validated with the film data, and Matlab algorithms and decision rules were developed to obtain for each child the pulling curve with the highest peak value and a duration of at least two seconds (unpublished data). Using the algorithm and decision rules two raters (L.R. and R.K.) independently rated all IMS measures.

Statistical analyses

For the typically developing infants descriptive statistics were used to characterize the study group for age, height and weight and PDI scores of the BSID-II. The inter-rater reliability of the algorithm to select the highest pulling action was determined by calculating the absolute agreement between two independent raters over all included measurements. In 50% of the participating children, the inter-rater reliability of the assessment procedure was determined by calculating the absolute agreement between two muscle strength assessments in the same participant by different assessors. For both reliability analyses a two-way random model was used to calculate ICC. Skewness and Kolmogorov–Smirnov test of normality were performed for infant muscle strength (IMS) and if appropriate IMS_{log} was calculated. Possible differences in IMS or IMS_{log} between boys, girls, and age groups were evaluated using analysis of variance (one-way ANOVA). The convergent validity of the IMS meter was evaluated by calculating Pearson's correlation coefficients between IMS and age, height, and weight. Regression analyses were performed to build a model for predicting muscle strength in typically developing infants using age, height, and weight as predictors. Prediction intervals for mean IMS, ± 1 and 2 SD were calculated. To compare the outcome of infants with PWS to the 24 months old typically developing infants, observed data of the infants with PWS are depicted in the developmental course based on the model. Moreover, the observed IMS was divided by IMS predicted multiplied by 100 (IMS%). Descriptive statistics were used to characterize the infants with PWS for age, height, weight, BSID-II raw scores, developmental percentage, IMS, IMS_{log}, and IMS%. Differences in age, height, weight, IMS, IMS_{log}, and IMS% between infants with PWS and typically developing infants were evaluated using analysis of variance (one-way ANOVA), and it was tested whether there is a relation between motor performance and muscle strength using Pearson's correlations. All analyses were performed using SPSS 19.0 for Windows.

Results

Participants

A total of 110 typically developing infants (59 boys and 51 girls) participated in this study. Twenty-nine infants were excluded: 16 infants refused to participate at the moment of testing because of tiredness or sickness (n=6), or they refused to sit in the IMS-meter (n=10), and 13 infants did not pull the toy during the pulling task. Characteristics of the 81 (74%) included infants are presented for each age group in Table 1.

Table 1. Characteristics and mean outcome measures of the typically developing infants presented by age group

	n	Gender M/F[a]	Age months (SD)	Height cm (SD)	Weight kg (SD)	BSID-II[b] PDI[c] (SD)	IMS[d] N[e] (SD)	IMS_{log}[d] N_{log}[e] (SD)
6-month-olds	11	7/4	6.2 (0.3)	68.9 (2.4)	8.3 (1.2)	99 (13.7)	29.5 (7.5)	3.4 (0.2)
9-month-olds	12	4/8	9.1 (0.4)	71.2 (2.0)	8.9 (1.2)	110 (15)	43.5 (16.9)	3.7 (0.4)
12-month-olds	13	7/6	11.9 (0.4)	73.7 (3.1)	9.1 (0.9)	100 (10)	47.0 (13.8)	3.8 (0.3)
18-month-olds	13	8/5	18.0 (0.5)	82.7 (4.1)	11.5 (1.3)	101 (7.7)	58.2 (30.1)	4.0 (0.5)
24-month-olds	14	7/7	23.9 (0.4)	88.6 (2.3)	13.0 (1.2)	109 (19.5)	94.1 (20.8)	4.5 (0.2)
30-month-olds	6	2/4	29.9 (0.2)	100.0 (4.5)	13.6 (1.1)	110 (15.9)	109.4 (32.6)	4.7 (0.3)
36-month-olds	12	6/6	36.0 (0.5)	99.5 (3.6)	15.1 (1.3)	100 (11.4)	136.6 (31.4)	4.9 (0.2)
Total	81	41/40	18.7 (10.1)	82.0 (11.2)	11.2 (2.7)	104 (14.1)	71.9 (42.5)	4.1 (0.6)

[a] M/F = male/female
[b] BSID-II = the Motor Scale of the Bayley Scales of Infant Development 2nd edition
[c] PDI = psychomotor developmental index
[d] IMS = infant muscle strength
[e] N = Newton

Motor performance was normal in all included infants. In the typically developing infants, age, height, weight, and BSID-II raw scores were highly correlated ($r \geq .86$) with each other. Twenty infants with PWS at 24 months of age participated in this study. Three were excluded: two did not pull the toy during the pulling task and one refused to sit in the IMS-meter. The characteristics of the 17 included infants with PWS and the 14 typically developing infants at 24 months of the age are presented in Table 2.

Table 2. Characteristics and mean outcome measures of the 24 months old infants with PWS and typically developing infants

	n	Gender M/F[a]	Age months (SD)	Height cm (SD)	Weight kg (SD)	BSID-II[b] RS[c] (SD)	IMS[d] N[e] (SD)	IMS_{log}[d] N_{log}[e] (SD)	IMS%[f] % (SD)
Typical	14	7/7	23.9 (0.4)	88.6 (2.3)[1]	13.0 (1.2)	86.2 (3.8)[2]	94.1 (20.8)[2]	4.5 (0.2)[2]	115.7 (25.3)[2]
PWS	17	10/7	23.7 (1.2)	85.1 (5.2)[1]	11.8 (2.3)	63.4 (6.7)[2]	59.1 (16.2)[2]	4.0 (0.3)[2]	79.1 (17.4)[2]

[1] $p<0.05$, [2] $p<0.001$: PWS vs. typical
[a] M/F = male/female
[b] BSID-II = the Motor Scale of the Bayley Scales of Infant Development 2nd edition
[c] RS = raw score
[d] IMS = infant muscle strength
[e] N = Newton
[f] IMS% = (IMS/IMS predicted) × 100

IMS inter-rater reliability

The inter-rater reliability of the IMS measurement procedure was tested. First the inter-rater reliability of the selection procedure to obtain the best pulling action for each participant was examined. For all 81 measurements two raters independently selected the best pulling action for each child using the Matlab algorithm and decision rules. Rater 1 analyzed a mean IMS of 71.9 Newton (SD = 42.5) and rater 2 a mean IMS of 72.2 Newton (SD = 42.9). The ICC was .99 ($p < .001$). In 46 participants a second assessor performed a second pulling task. Twenty-five children of different ages performed the pulling task a second time. The ICC was .84 ($p < .001$). Assessor 1 measured a mean IMS of 98.3 Newton (SD = 50.0) and Assessor 2 a mean IMS of 89.9 Newton (SD = 50.6). Taken into account the large variation in age, the difference in mean value is small and SD comparable. As the order of the assessors was altered, the mean IMS of the first and second measurement was calculated as well. IMS-1 was 93.5 Newton (SD = 48.9), IMS-2 was 94.7 Newton (SD = 52.0), the ICC was .83 ($p < .001$).

Convergent validity

IMS correlated highly with age, height, weight and BSID-II raw scores in typically developing infants (Table 3), which confirms the convergent validity of the IMS-meter.

Table 3. Pearson's correlations between IMS, age, height, weight, and raw scores of the BSID-II in typically developing infants

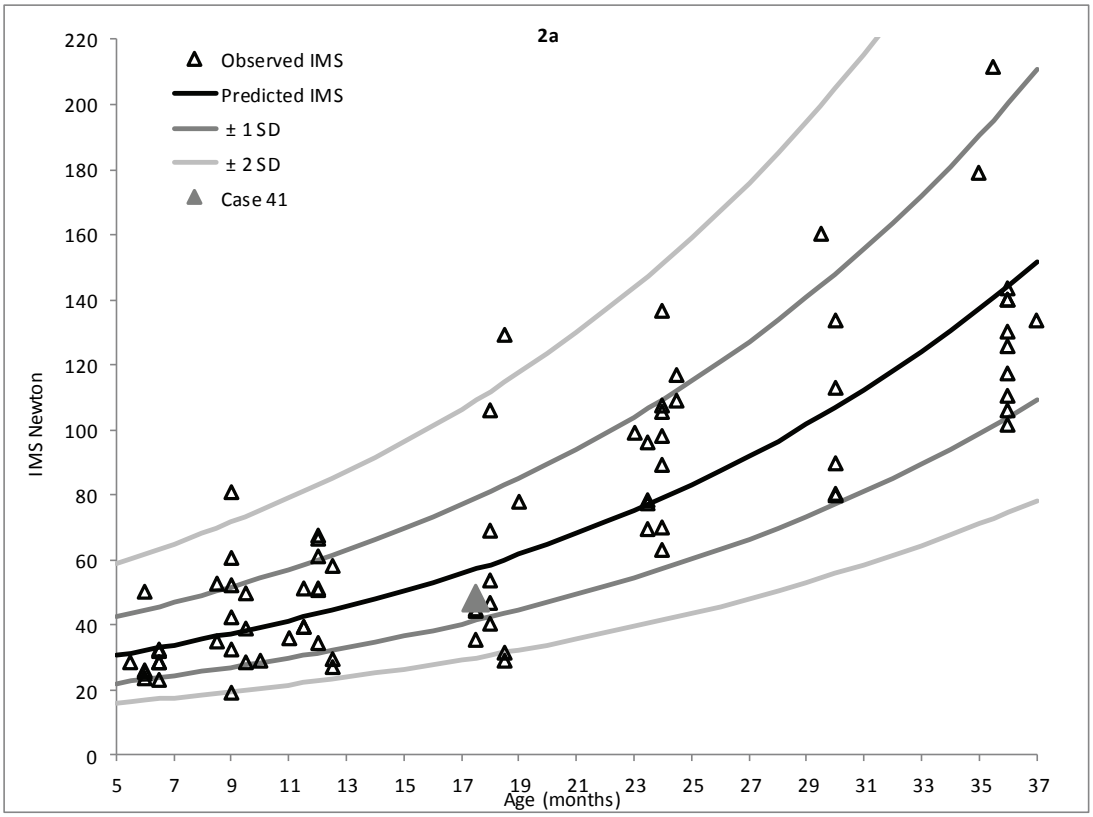
	Age (months)	Height (cm)	Weight (kg)	BSID-II[a] (raw scores)
IMS_{log}[b] ($Newton_{log}$)	.85	.85	.81	.83
IMS[b] (Newton)	.84	.84	.79	.80
Age (months)	-	.96	.90	.96
Height (cm)		-	.94	.93
Weight (kg)			-	.86

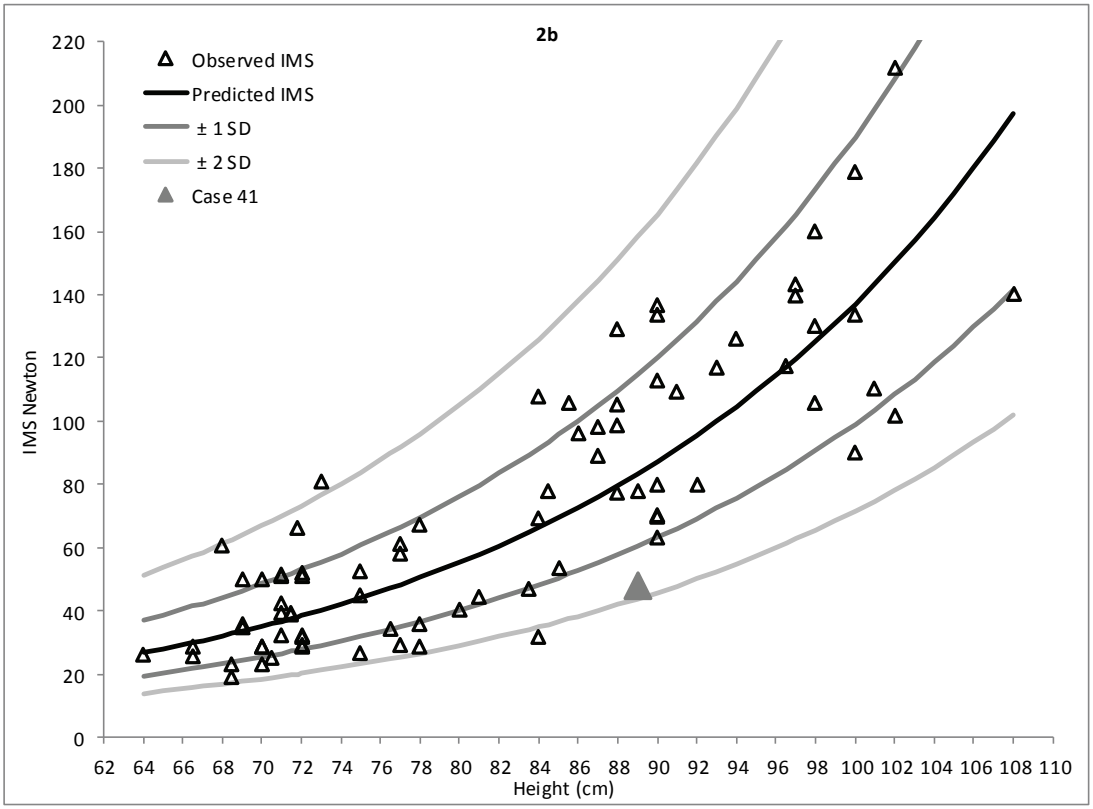
[a] BSID-II = the Motor Scale of the Bayley Scales of Infant Development 2nd edition
[b] IMS = infant muscle strength

IMS results of typically developing infants

Since the IMS measures were not normally distributed (skewness of .91 and a significant Kolmogorov–Smirnov test ($p < .001$)) the data were transformed to natural logarithms. The IMS_{log} was normally distributed ($p = .16$). IMS and IMS_{log} differed significantly over age groups ($F(6, 74) = 33.7$, $p < .001$, and ($F(6, 74) = 35.9$, $p < .001$); post hoc test specified that IMS differed between: 6 versus ≥ 18-month olds, 9 versus ≥ 24-month olds, 12 versus ≥ 24-month olds, 18 versus ≥ 24-month olds, and 24 versus 36-month olds. The results for IMS_{log} were similar except for 6-month olds, which differed significantly from all older age groups. There were no gender differences in muscle strength. Table 1 presents the mean and SD of IMS and IMS_{log} per age group. IMS_{log} was used in the linear regression analyses since the logarithmic IMS was normally distributed, did not contain influential cases, and improved the homoscedasticity and linearity. Afterwards IMS_{log} was transformed back into IMS so that the outcome measures could be presented in Newton. Simple

linear regression analyses revealed that height accounted for the greatest percentage of the explained variance in IMS_{log} (72%), followed by age (71%) and weight (65%) (Figure 2).





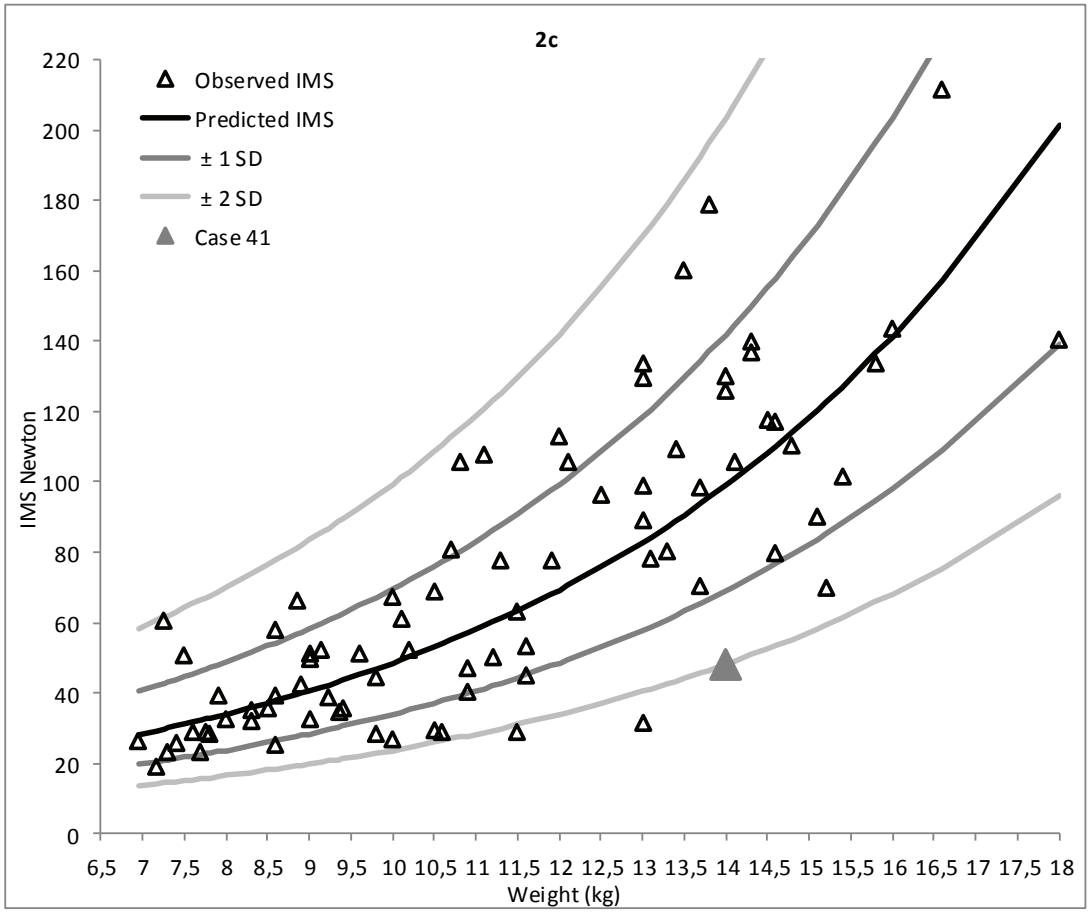


Figure 2. IMS as a function of age (2a), height (2b), or weight (2c)
Lines present mean predicted IMS (black line), prediction intervals for ± 1 and 2 SD (grey lines), observed IMS (triangles), and case 41 (large grey triangle)

Based on multiple linear regression analyses, a model was build to predict muscle strength in infants, in which IMS_{log} was taken as the dependent variable and age, height, and weight as predictors. Together they accounted for 73% of explained variance. Since age, weight, and height are strongly related, multicolinearity existed. Therefore, the predictors did not make a significant unique contribution to the prediction of muscle strength in the model. Taking the standardized coefficients into account, the contribution of age and height to the prediction of muscle strength were similar (ß = .39 versus .38) while the contribution of weight was much smaller (ß = .10). Using reference values, Figure 3 shows the IMS prediction lines based on age, height, and weight, for typically developing boys and girls with normal height and weight according to age.[25]

IMS in infants with Prader–Willi syndrome

Age was similar in both groups, and as expected, infants with PWS were significantly smaller. Although not significant (p = .08), weight also seemed to be somewhat lower in infants with PWS (Table 2). IMS, IMSlog, and IMS% were significantly decreased in infants with PWS compared to typical 24 months olds, as well as BSID-II raw scores (Table 2). Figure 3 shows IMS in infants with

PWS compared to typically developing infants of 24 months old and the prediction of IMS based on the model.

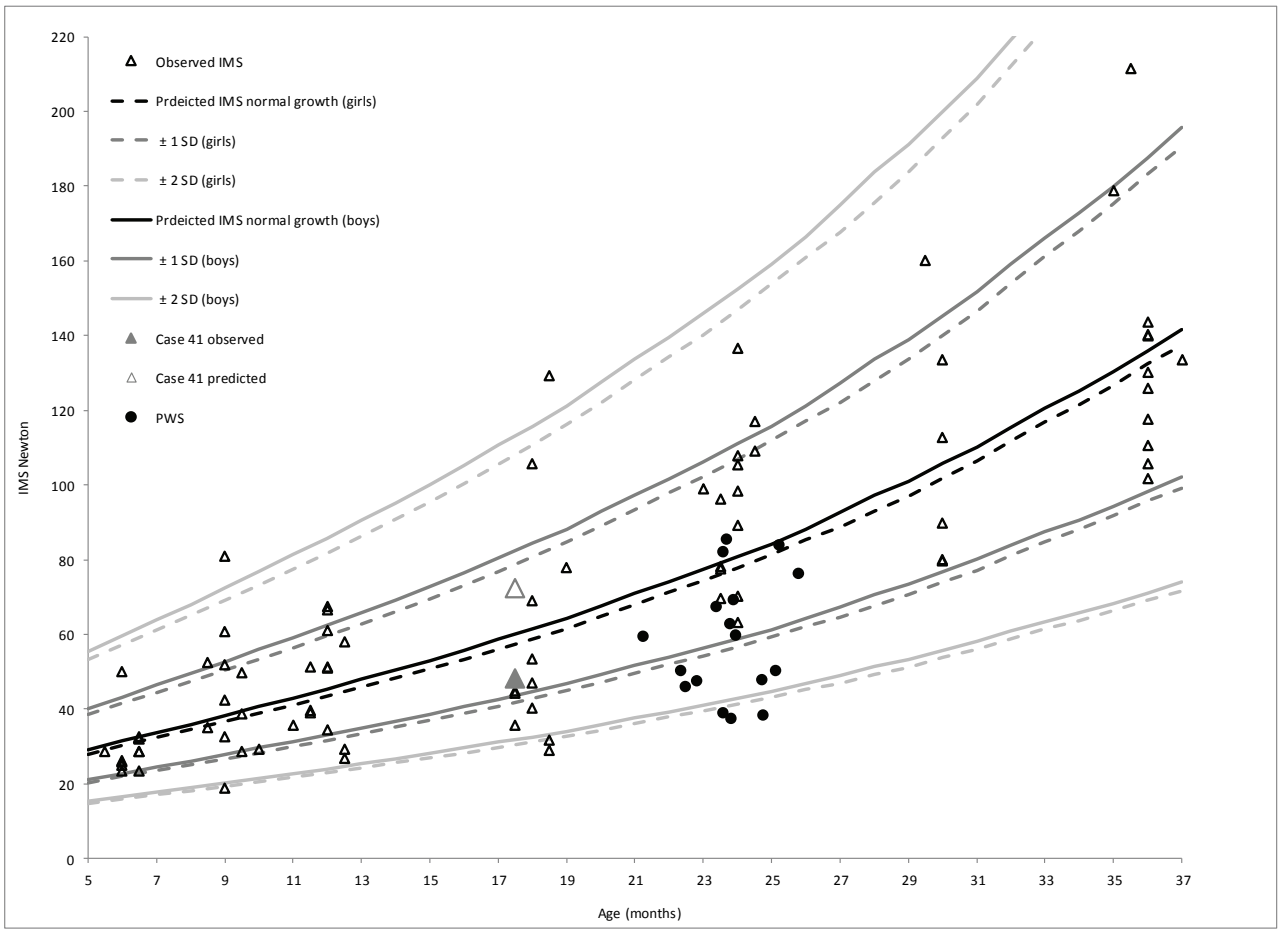


Figure 3. IMS as a function of age, height, and weight
Lines present mean predicted IMS of infants with normal growth (boys = solid, girls = dashed), together with prediction intervals ± 1 and 2 SD, observed IMS (triangles), Case 41 (large grey triangles: open = predicted, closed = observed) and observed IMS in 24 month old infants with PWS (black circles).

Motor developmental percentage in infants with PWS was on average 55.3% (SD = 9.0, range 42.5–76.0%). There was a positive correlation between IMS and motor performance in infants with PWS (Figure 4). Stronger infants scored higher on the BSID-II and had a higher developmental percentage ($p < .001$, $r = .75$ and $p < .01$, $r = .70$). In typically developing infants there was no correlation between IMS and motor performance.

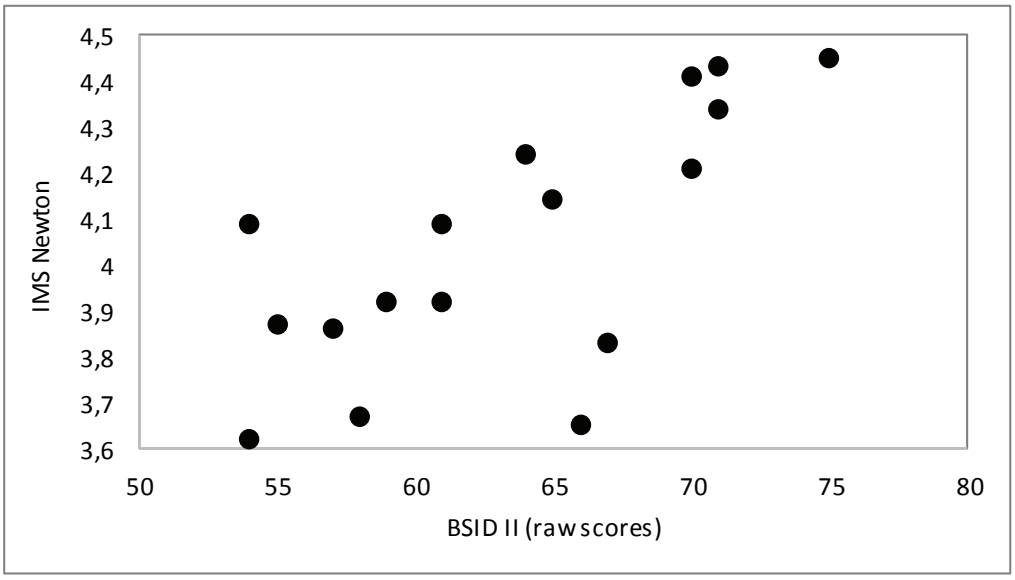


Figure 4. Correlation between IMS and BSID II (raw scores) in 24 months old infants with PWS
p<.001, r = .75.

Discussion

As far as we know, this is the first study to objectively measure muscle strength in infants and toddlers. Muscle strength was measured using a pulling task, attuned to the natural behavior of young children, from which the maximal total generated strength during pulling was obtained. We concluded that the task is suited provoking infants to show their muscle strength, since 74% of the infants performed the pulling task correctly, a relatively high percentage given their young age. The inter-rater reliability of the IMS-meter was good (ICC = .84) and on the same level as what is reported for hand-held dynamometry.[8,16] Moreover, IMS scores in infants with PWS were significantly lower than in typically developing infants and in contrast to typically developing infants, IMS was significantly related to motor development. The IMS measurement helped us to objectify low muscle strength as an underlying cause of motor developmental problems in infants with PWS.

Because we expected a high number of refusals due to the young age of the infants, 46 of the infants (about 50%) were measured a second time. Twenty-five infants performed the task a second time and this number was sufficient to determine that inter-rater reliability is good (ICC = .84). Moreover, the results of the first and second measurement per infant were highly comparable (ICC = .83), which implies that infants and toddlers reproduce a similar maximum strength a second time. It appeared that choosing the highest peak value out of several pulling actions performed during the pulling task is valid considering the high correlation between the first and second measurement. This method is also used in hand-held dynamometry.[18,19,26] Muscle groups, like the arm flexors or leg extensors, are usually measured separately in muscle strength tests. However, in this study we measured the total generated strength in a pulling task, as infants are not able to activate single muscle groups. Since in normal healthy individuals grip

strength is highly related to the strength of other muscle groups[27] and in children with PWS no selective muscle strength impairment is expected (as in hemiparesis, spina bifida, or neuromuscular disease), we presume that the pulling strength is representative for general muscle strength. Furthermore, given the fact that muscle strength needs to be adapted to functional biomechanical demands, high correlations with anthropometry and age were expected because of earlier muscle strength studies in older children and adults.[13,14,16,19,28,29] This strong correlation was indeed found, as muscle strength is highly related to age, height, and weight in infants and toddlers. This confirmed the convergent validity of the IMS-meter. Age and height were the most predictive factors for muscle strength, which is also reported in the study of Rose et al.[16] on muscle strength in children aged 30 months. Conversely, in older children age and weight are usually reported as most predictive,[13,18,19] and from puberty gender becomes an important predictor.[18,19] In adults, gender, age, weight, and height remain important predictors for muscle strength, although the correlation is weaker than in infants and children.[27-31] Presumably, because variation in muscle strength increases later on in life, as a consequence of training and physical activity.

Age, height, and weight were each found to be reasonable individual predictors of muscle strength in infants and toddlers. Theoretically, however, age, height and weight have different influences on muscle strength and the age-related variation in height and weight in infant and toddlers is high, especially in patient groups. We therefore concluded a prediction of muscle strength based on all three factors would be more accurate. To illustrate the risk of misinterpretations, we will discuss Case 41 from our sample (Box 1).

Box 1: Prediction of muscle strength, based on one predictor versus all three predictors

Case 41 is a girl aged 17.5 months. She is 89 cm in height and weighs 14 kg. She is tall in relation to her age (Standard Deviation Score for height = +2.4 SD). When the model is used to predict muscle strength, the following calculation has to be performed:

IMS_{log}' = 1.75121226 + (.023066202 × age) + (.020516112 × height) + (.021364952 × weight), which is transformed back into predicted IMS by taking the antilogarithm of the predicted IMS_{log} value.

For Case 41 we calculate muscle strength predicted on:

	IMS' (Newton)	**IMS' -1 SD** (Newton)	**IMS' -2 SD** (Newton)
Age, height, and weight	72.2	51.8	37.2
Age	57.1	41.4	29.9
Height	83.3	60.5	43.9
Weight	69.0	54.3	48.1

The observed IMS of Case 41 is 48.2 Newton. This is within the lower normal range when all three predictors are taken into account, normal when only age is taken into account, and near -2 SD when only height or weight are taken into account. See Figures 2 and 3 in which Case 41 is highlighted.

The model to predict muscle strength based on age, height, and weight seems very accurate, because it accounted for 73% of explained variance. However, because a model makes the best

fit over all observations and the variance in IMS between children with the same age, height and/or weight is quite large (Figures 2 and 3), the prediction for IMS in a infant with a specific age, height and weight can be less optimal. For example, as shown in Figure 3, at 36 months of age, most of the observed data lies below the prediction line, and therefore the prediction line is pulled a little bit downwards. This improves the prediction in 36 month old infants but underestimates the prediction in younger children. To overcome these problems more reference data is needed to optimize the model.

We also compared muscle strength of typically developing infants of 24 months old to 24 month old infants with PWS. Seventeen of the 20 infants with PWS performed the pulling task correctly, which is a high number considering their young age and seriously motor developmental delay. As expected, muscle strength and motor performance was significantly decreased in infants with PWS. On average they had a IMS% of 79.1%, but because the high individual variation, there were also infants with PWS who reached normal muscle strength (Figure 3). Infants with PWS who reached normal muscle strength also had a relatively higher motor developmental level (Figure 4). Moreover, because typically developing infants scored in average at 115%, we can conclude that the current model underestimates predicted muscle strength at the age of 24 months and therefore the IMS% scores in PWS are overestimated.

In this study, in infants with PWS, IMS% was higher than expected related to their motor developmental level and related to the finding that in adults with PWS muscle strength of the knee flexor is 70% decreased.[32] Alternatively, in individuals with PWS muscle mass is 25–37% decreased,[33] and because of a rough linear relation between muscle strength and muscle cross-sectional area, a decrease in muscle strength of about 25–37% is likely.

In typically developing infants, the absence of a relationship between muscle strength and motor performance indicates that muscle strength is sufficient enough to perform all age related skills. At the age of 24 months muscle strength is presumably higher than needed for the functional skills and the higher variation in muscle strength in typically developing children is not a restricting factor in functional skills. However, in children with PWS, there was a high correlation between muscle strength and motor performance. This indicates that in infants with PWS muscle strength seems to be a determining factor for skill acquisition, especially in case of antigravity movements.[34] At this point muscle strength seems to be an important factor for motor developmental delay in infants with PWS, but future more in depth studies are needed to confirm this.

Taken together we were able to determine muscle strength in young infants between six and 36 months in a reliable, objective, and reproducible way. Moreover, we could determine that muscle strength is a restrictive factor on motor development in PWS. However, more IMS reference data are needed to optimize the IMS prediction model. Furthermore, the IMS meter needs to be further explored in other patient groups between six and 36 months of age and technical improvements are needed for use in clinical practice.

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6

Growth hormone improves motor development in infants with Prader-Willi syndrome by increasing muscle mass

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Abstract

Objective: To investigate the effect of training combined with growth hormone (GH) on muscle thickness, and its relationship with muscle strength and motor development in infants with Prader-Willi syndrome (PWS).

Methods: In a randomized controlled trial twenty-two PWS infants (12.9 months ± 7.1) were followed over two years comparing a treatment (n=10) and a waiting list control group (n=12). Muscle thickness of four muscle groups was measured using ultrasound. Muscle strength was evaluated using the Infant Muscle Strength meter. Motor performance was measured with the Gross Motor Function Measurement. ANOVAs were used to evaluate between group effects of GH on muscle thickness at six months and to compare pre- and post-treatment (after 12 months GH treatment). Multilevel analyses were used to evaluate effects of GH on muscle thickness over time and multilevel bivariate analyses were used to test relationships between muscle thickness, muscle strength, and motor performance.

Results: A significant positive effect of GH on muscle thickness ($p < .05$) was found. Positive relationships were found between muscle thickness and muscle strength ($r = .61$, $p < .001$), muscle thickness and motor performance ($r = .81$, $p < .001$) and muscle strength and motor performance ($r = .76$, $p < .001$).

Conclusions: GH increased muscle thickness, which was related to muscle strength and motor development in PWS infants. Catch-up growth was faster in the muscles that are most frequently used in early development. As this effect was independent of GH it suggests a training effect.

Introduction

Prader-Willi syndrome (PWS) is a multisystem disorder with an estimated prevalence of 1 in 10,000-30,000 live births[1]. The syndrome results from lack of expression of the paternally derived chromosome 15q11-q13, caused by a deletion[2], uniparental disomy,[3] imprinting center defect, or balanced translocations.[4] PWS is characterized by hypotonia, short stature, hyperphagia, obesity, mild dysmorphic facial features, cognitive and behavioral deficits and by endocrine disturbances like hypogonadism and growth hormone (GH) deficiency.[3,5] In infancy, severe hypotonia combined with muscle weakness leads to serious motor developmental delay.[5-9] These motor problems persist, although they are less marked, in childhood and adulthood.[10-16] It is presumed that the motor problems are related to an increased fat-muscle ratio even in underweight PWS infants.[17-19] In PWS infants, body fat percentage ranges from 28-32%, increasing to 36-55%[21-23] during childhood; in developmentally normal infants it is 24%, decreasing to 18% during childhood.[17,18,20] In children and infants with PWS body fat percentages decreases as result of GH treatment,[18,20,21,24-26] although the fat-muscle ratio does not normalize.[27] Dual Energy X-Ray Absorptiometry showed a positive GH effect on Lean Body Mass (LBM), which mainly contains muscle tissue[19-22,24,26-28]. In PWS infants GH positively influences motor development.[8,9,20] In children with PWS GH treatment has a positive effect on agility and thoracic muscle strength.[21,26,29,30]

We hypothesize that GH improves muscle strength and motor development by increasing muscle mass in PWS infants. This is the first longitudinal study focusing on the effect of GH on muscle thickness - measured with ultrasound - and the relation between muscle thickness, muscle strength and motor development in PWS infants.

Methods

Design

This study was part of a two-year randomized single-blind controlled trial focused on motor development in PWS infants.[9] All infants received physical training, and after stratification for age, PWS infants were randomized (1:1) either to the GH group, in which infants were treated with 1mg/m^2/day GH (Genotropin®, Pfizer), or to the control group, in which GH treatment started after an initial control period (Figure 1). Randomization was done by the Dutch Growth Research Foundation (DGRF), using a computer-generated list of random numbers. Originally we planned a control period of 12 months, however, the results of Festen and colleagues[8] demonstrated the effectiveness of GH so we shortened the control period to six months on ethical grounds. An extra measurement three months after baseline was added to provide three measurements in the control condition. During the two-year study period muscle ultrasound measurements were taken at six-month intervals, and muscle strength and motor performance were assessed at three-month intervals. The study leader (M.N.), researcher (L.R.), the electrodiagnostic technicians performing the muscle ultrasound scans (H.J., W.R., and J.B.) and the pediatric physical therapists (A.Z. and I.D.) were all blind to the group assignment of subjects.

All parents gave written informed consent and the study was approved by the Medical Ethics Committees of the Erasmus Medical Centre Rotterdam and Radboud university medical center.

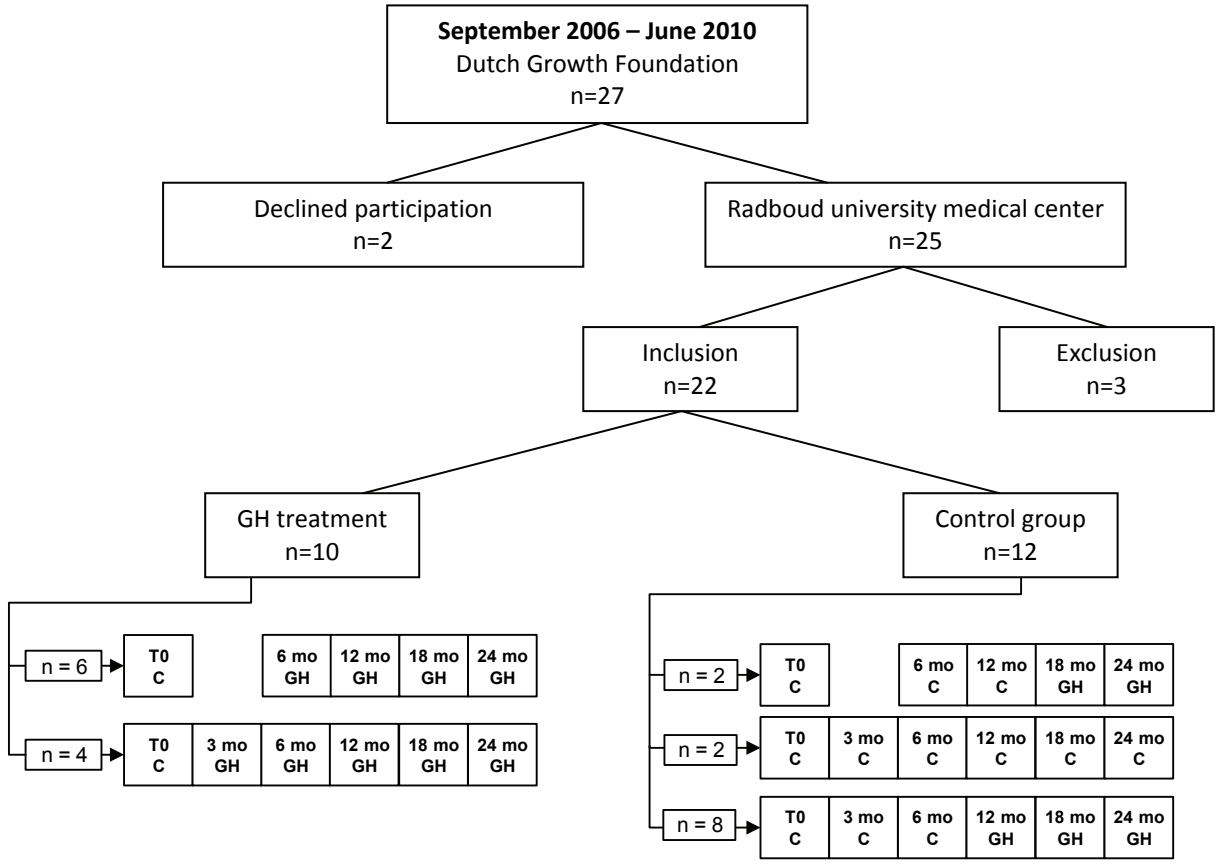

Figure 1. Flowchart subject selection, inclusion and distribution among two groups
C = control observation, during the preceding period the infant had not received GH treatment,
GH = treatment observation, during the preceding period the infant had received GH treatment.

Participants

All parents of PWS infants who were registered at the DGRF between September 2006 and June 2010 - the majority of PWS infants in the Netherlands diagnosed within that period - were invited to participate. The patient recruitment procedure has been previously reported.[9] Of twenty-seven potential participants, two did not want to participate, three infants were excluded and parents of two infants refused GH treatment, but wanted to participate in the training, these infants were added to the control group without randomization. Hence, 20 infants were randomly assigned: 10 to the GH group and 10 to the control group, giving final group sizes of GH = 10 and control = 12. (Figure 1 and Reus et al.,[9]). Mean age at the start of study was 12.9 months (±7.1, range: 4.7-31.8) and mean age at the start of GH treatment was 17.5 months (±7.3; range: 6.7-34.2). The clinical characteristics of the subjects and genetic subtypes are presented in Table 1.

Table 1. Clinical characteristics at baseline for all infants, the control group and the GH-treated group

n	All subjects n=22 Mean (SD)	Control group n=12 Mean (SD)	GH group n=10 Mean (SD)	Control group vs. GH group *p*-value
Gender (M/F)	14/8	9/3	5/5	.44
Ethnicity (Dutch/non-Dutch[a])	19/3	10/2	9/1	.57
Genetic subtype (Deletion/UPD[b]/unknown)	10/9/3	5/4/3	5/5/0	1.0
Age in months	12.9 (7.1)	11.7 (6.3)	14.2 (8.1)	.43
Age at start of GH in months	17.5 (7.3)	18.0 (6.6)	17.1 (13.6)	.85
Height in SDS	-1.8 (1.2)*	-2.0 (1.1)	-1.6 (1.2)	.40
Weight in SDS	-1.3 (1.5)*	-1.6 (1.5)	-0.9 (1.6)	.29
Muscle thickness in SDS:				
Biceps brachii	-1.6 (0.7)*	-1.5 (0.7)	-1.7 (0.7)	.50
Forearm flexors	-1.3 (1.0)*	-1.2 (0.9)	-1.5 (1.1)	.55
Quadriceps	-1.6 (0.9)*	-1.8 (1.0)	-1.3 (0.8)	.23
Tibialis anterior	-1.5 (0.5)*	-1.5 (0.5)	-1.4 (0.5)	.81
Muscle strength:[c]				
IMS in Newtons	28.9 (14.1)	26.7 (10.1)	31.3 (17.7)	.47
IMS%[d]	58.1 (22.4)	54.8 (14.3)	61.7 (28.1)	.50
Motor performance:				
GMFM total score	26.8 (18.9)	23.7 (18.8)	30.4 (19.3)	.43

* *SDS significantly below 0* ($p \leq .001$)

[a] All infants were born in the Netherlands, but 3 families came from the Middle East.

[b] UPD = uniparental maternal disomy

[c] Muscle strength could not be measured in all infants at baseline, because at the start of the study not all infants were able to perform the pulling task used to measure muscle strength owing to motor developmental delay. We have therefore reported the results of the first measurement done at a mean age of 17.2 (±7.0) months, which is in most infants, the first or second measurement of the trial.

[d] IMS % = (observed IMS/predicted IMS) × 100

Outcome measures

Muscle measurement

Muscle thickness and muscle echo intensity of the left biceps brachii, right forearm flexors, right quadriceps and left tibialis anterior muscle were measured using ultrasound. This technique shows high reliability and reproducibility in measuring muscle thickness when compared to MRI.[31,32] The measurements were performed by three well-trained electrodiagnostic technicians (H.J., W.R., and J.B.) using an IU22 ultrasound device (Philips, Best, The Netherlands) with a linear broadband 17-5 MHz transducer. Measurements were made at fixed anatomically defined positions as described in a previous study.[33] All muscle thickness and muscle echo intensity data were expressed as z-scores (i.e. the number of standard deviations above or below normal) compared to weight-specific reference values.[33] Echo intensity scores were considered abnormal if the z-score exceeded 3.5 SD in one muscle group, or 2.5 SD in two muscle groups, or 1.5 SD in three muscle groups.[34] Z-scores for each muscle group and an overall average muscle thickness score (sum of muscle thickness per muscle group divided by four) were used as outcome measures.

Muscle strength and motor performance

Muscle strength was assessed using the "Infant Muscle Strength meter" (IMS meter), in which a pulling task is used to evoke maximal pulling activity. The IMS meter is a new, reliable and valid measurement method for measuring muscle strength objectively in infants from 6-36 months.[35] Muscle strength can be assessed from the moment the infant is able to sit with support, reach and grab an object, typically from six months of age. IMS% was calculated using reference data from a prediction model[35] by dividing observed IMS by predicted IMS (based on age, height and weight). Both IMS and IMS% were used as outcome measurements.

Motor performance was assessed using the Gross Motor Function Measurement (GMFM)[36]. This test contains 88 items grouped into five dimensions (e.g. lying and rolling, sitting, crawling and kneeling, standing, walking, running and jumping)[9] and is sensitive to motor developmental changes over time in PWS infants. Typically children can perform correctly on all eighty-eight items at the age of five years. Each item is scored on a four-point ordinal scale, and a percentage total GMFM score was calculated by dividing the sum of the actual item scores by the possible maximum score. This score was used as an outcome measure. All assessments of muscle strength and motor performance were performed by two well-trained pediatric physiotherapists (A.Z. and I.D.).

Statistical analyses

Descriptive statistics were used to characterize and compare the groups at baseline. A binomial test was used to compare z-scores in PWS infants with developmental norms with respect to height, weight, and muscle thickness. ANOVA was used to compare muscle thickness z-scores between groups after six months (GH vs. control), and to compare pre-treatment scores with scores after 12 months of GH treatment (both groups). Multilevel regression analyses (MLRA) were used to evaluate muscle thickness z-scores over time, taking both within-subject variance (level one) and between-subject variance (level two) into account. This technique is well suited to the analysis of data related to growth.[37] For each muscle group, a regression model was developed to predict muscle growth using age, baseline muscle thickness and GH as explanatory variables. We tested model fit by calculating the proportional reduction in unexplained variance between a model with no explanatory variables (empty model) and the final models.[38] Multilevel bivariate analyses were used to calculate inter-class correlations between the average muscle thickness of four muscle groups, muscle strength (as IMS score), and motor performance (as GMFM score). MLRA was performed using "lmer" in the software package R; other statistical analyses were performed using SPSS 21.0.

Results

Baseline results

The groups did not differ in terms of clinical characteristics at baseline (Table 1). Height, weight and muscle thickness of biceps brachii, forearm flexors, quadriceps, and tibialis anterior, all expressed as standardized deviation score (SDS), were significantly lower than in healthy peers. All muscle echo intensity measurements were normal. Muscle strength was 58% of predicted muscle strength for healthy peers corrected for age, height and weight, which is comparable with previously reported motor developmental outcomes in PWS (55% of normal reference)[9].

Muscle ultrasound results

After six months, the forearm flexors muscles were significantly thicker in the GH group than the control group (Table 2). Furthermore, after six months muscle thickness in the GH group had improved significantly in the biceps brachii, forearm flexors, and tibialis anterior compared with baseline, whilst in the control group only the muscle thickness of the tibialis anterior had improved (Table 2).

After 12 months of GH treatment muscle thickness in the forearm flexors and quadriceps was significantly improved in both groups and thickness of the biceps brachii and tibialis anterior muscles had increased in the GH group (Table 2).

Table 2. Standardized deviation score (SDS) of muscle thickness in the control and GH groups at baseline, at 6 months (GH vs. control) and after 12 months of GH treatment in both groups

	Baseline		**T2 (6 months control or GH)**		**GH 12 months (Both groups 12 months GH)**	
	Control group	GH Group	Control group	GH Group	Control group	GH Group
Biceps brachii	-1.5 (0.7)	-1.7 (0.7)	-1.4 (0.8)	-0.5 (1.7)^	-0.7 (0.9)	-0.6 (1.0)^^
Forearm flexors	-1.2 (0.9)	-1.5 (1.1)	-1.3 (0.8)*	-0.5 (0.9)*^	-0.3 (0.6)^^	-0.1 (1.0)^^
Quadriceps	-1.8 (1.0)	-1.5 (0.9)	-1.4 (0.9)	-0.9 (1.4)	-0.4 (0.7)^^	-0.5 (1.0)^^
Tibialis anterior	-1.5 (0.5)	-1.6 (0.5)	-0.8 (0.6)^	-0.6 (1.1)^	-0.8 (1.1)	-0.8 (0.9)^^

* Between group difference $p < .05$

^ Baseline vs. T2 $p < .05$

^^ pre-treatment vs. 12 months GH $p < .05$ In the GH group pre-treatment is baseline, in the control group pre-treatment is the final observation of the control period.

Development of muscle thickness (MLRA models)

We also evaluated the effect of GH over time using MLRA. In total, 118 muscle ultrasound scans were analyzed (in eight infants five repeated scans and in 14 infants six repeated scans is 124 – 6 missing), of which 52 were control observations. Models for all four muscle groups demonstrated a significant positive effect of GH on muscle thickness, when controlled for age and baseline muscle thickness (Table 3 and Figure 2). The models predicted the same effect independent of the age at which GH treatment started. In the biceps brachii and tibialis anterior models, muscle thickness did not change over time (no age effect), indicating that growth rate was similar to typical development although GH treatment enhanced the effect on muscle thickness (Table 3

and Figure 2a, 2d). In the quadriceps and forearm flexors models, muscle thickness did improve (a significant positive age effect; Table 3 and Figure 2b, 2c), indicating that growth rate was increased compared to typical development and GH accelerated the process of catching up by increasing muscle thickness. Moreover, a significant effect of baseline in all four models suggested that differences in muscle thickness between subjects were at least partly determined by individual differences at baseline (Table 3). This baseline effect varied between muscle groups: the biceps brachii and tibialis anterior were initially thicker and remained consistently thicker over time; the forearm flexors and quadriceps muscles showed a faster growth rate in infants with initially smaller muscles (significant negative interaction effect, Table 3), indicating a catch-up over time particularly in infants who had thinner muscles at baseline.

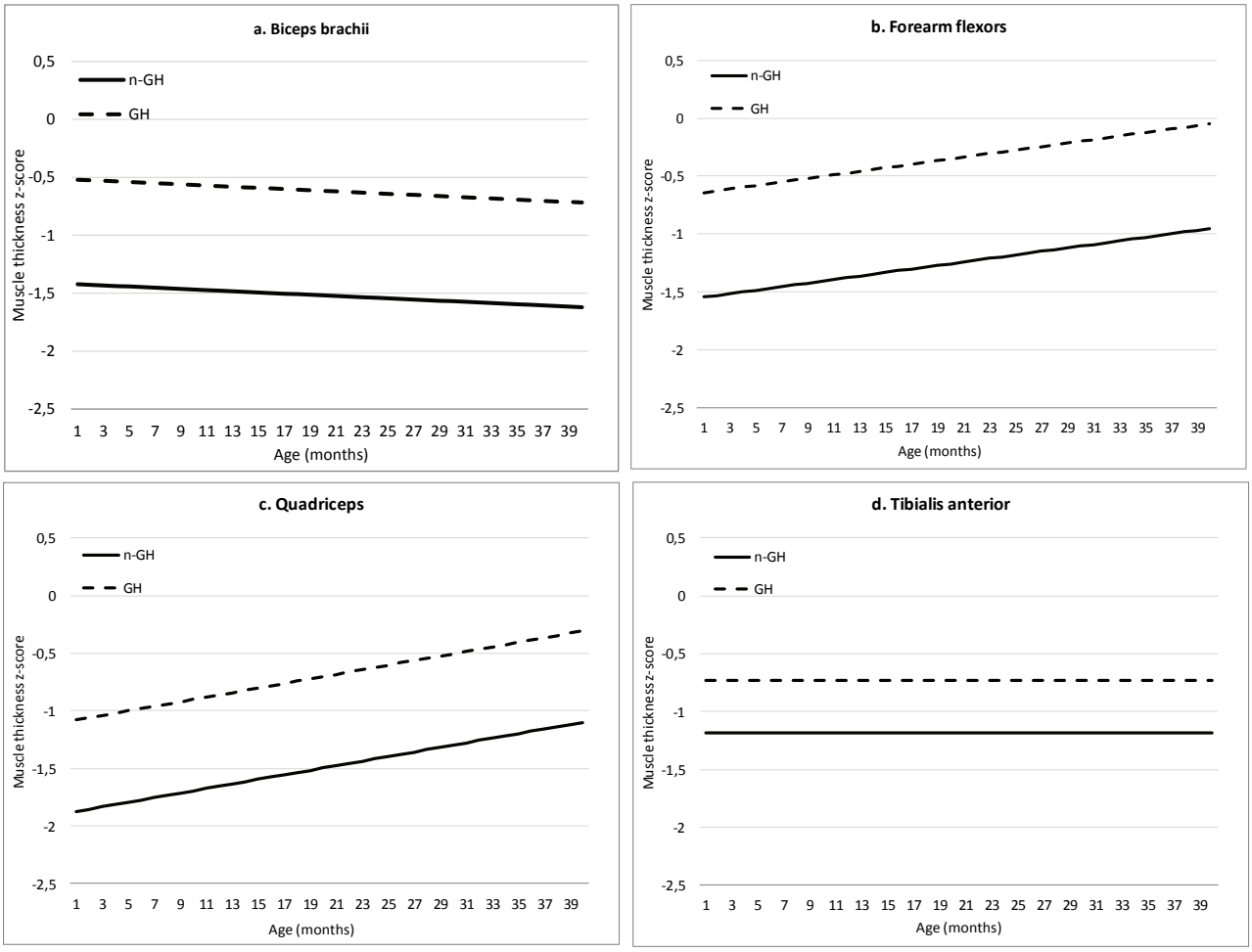

Figure 2. Average predicted muscle thickness development with GH treatment (dotted line) and without (solid line).

Assessing the fit of the model for muscle development by comparison with observed data showed proportional reduction of unexplained variance varied between 41% and 71%, which indicating a moderate to good fit (Table 3).

Table 3. Results of the four multi-level regression models for muscle thickness development, including the explanatory variables (age, GH, and baseline muscle thickness) and the interactions

	Biceps brachii	Forearm flexors	Quadriceps	Tibialis anterior
Intercept	-3.993	-7.522	-6.673	-3.584
Age	-0.005	0.163**	0.162**	0.000
GH	0.902***	0.906**	0.794 **	0.455*
Baseline average muscle thickness	3.442**	5.847 **	3.420**	3.459**
Age × Baseline average muscle thickness	-	-0.145**	-0.102**	-
Between subject variance intercept	0.018	0.002	0.200	0.060
Between subject variance age	0.001	0.000	0.000	0.007
Within-subject variance	0.67	0.28	0.65	0.39
Variance empty model	1.14	0.95	1.28	0.79
Proportional reduction in unexplained variance[a]	41%	71%	49%	51%

*p < .01, **p < .001

[a] The reduction of the unexplained variance when the empty model is compared to the final model.

Explanatory variables: Age = age in months; GH = a Boolean variable (0 = no, 1 = yes); Baseline average muscle thickness = sum of muscle thickness per muscle group divided by 4.

Relationship between muscle thickness, muscle strength, and motor performance

From the 118 planned IMS measurements 25 measurements were missing because infants had not yet mastered the required pulling skills to perform the test and five measurements were missing because the infants refused to perform the test, so 88 IMS measurements were available for analysis. One GMFM measurement was missing, so 117 GMFM measurements were available for comparative analysis. IMS and GMFM were measured three-monthly, so 154 simultaneous observations were made, seven repeated measurement sets per infant on average. Inter-class correlation between muscle thickness and muscle strength was r = .61 (p < .001), between muscle thickness and motor development r = .81 (p < .001) and between muscle strength and motor development r = .76 (p < .001).

Discussion

This study showed that muscle thickness was significantly decreased in the biceps brachii, forearm flexors, quadriceps and tibialis anterior muscle; with normal muscle structure (as measured by muscle echo intensity).[34] We demonstrated for the first time that in infants with PWS, decreased muscle thickness is strongly associated with decreased muscle strength and motor performance. GH treatment combined with physical training significantly increased muscle thickness, and this was matched by an increase in muscle strength and motor development.

Our finding of decreased muscle thickness in infants with PWS is in line with the reported lower LBM in PWS infants[17-20] and the early findings of type-2 muscle fiber atrophy and smaller type-1 muscle fiber size in infants with PWS.[39] Some studies reported that GH increases LBM (mainly determined by muscle mass),[20-22,26,29] however, in these studies the interpretation of the reported results is problematic because the increase in LBM was not corrected for changes in height. Studies in children with PWS that report height-corrected LBM have found that LBM normally decreases over time, but with GH treatment LBM stabilizes.[24,27,40] In contrast to in later childhood, GH treatment in infancy leads to an improvement in height-corrected LBM.[19] Our study results confirm these findings.

Another interesting finding is that muscle thickness at baseline varied widely between infants, which is in accord with clinical observations that hypotonia and muscle strength also show considerable variation. This might be related to innate or prenatal predispositions. Future studies with larger groups should focus on the relationship between muscle thickness and motor development in relation to chromosome 15q11-q13 deletion, uniparental disomy, imprinting center defect, or balanced translocations.

In this study changes over time also showed differences between the biceps brachii and tibialis anterior and the forearm flexors and quadriceps. In the former muscle groups, muscle growth rate was in line with muscle growth in developmentally normal infants, although at a lower level during the physiotherapy-only period. The GH treatment led to a spike in the rate of growth in muscle thickness which leveled out subsequently. In these muscle groups, infants with smaller muscles at baseline continued to have relatively smaller muscles throughout the study. In the forearm flexors and quadriceps however, catch-up growth was observed even during the physiotherapy period; this manifested as an increase in growth rate compared to muscle growth in developmentally normal infants, and GH treatment accelerated this process further. In these muscle groups, growth rate was higher for infants whose muscles were thinner at baseline. We hypothesize that the observed differences in catch-up growth between muscle groups are related to the degree to which these muscles are used in daily life, which is in turn related to the order of motor skill acquisition in developmentally normal infants and infants with PWS. Acquisition of the first fundamental skills during early motor development relies more on use of the forearm flexors and quadriceps than the biceps brachii and tibialis anterior.[41] After the typical severe hypotonic phase PWS infants demonstrate more spontaneous movements.[6] However, the order differs from typical infant development: head control for instance is easier in a vertical position than in a horizontal lying position, and the infants start to reach and grasp while using a supporting surface to overcome the influence of gravity. The forearm flexors are used more than the biceps brachii for the manipulation of objects, because infants use pronation of the forearm frequently but do not yet lift objects. The quadriceps and foot extensors are used when infants learn to push themselves forward across the floor and to stand and walk. In the early phase of walking, infants do not use dorsal flexion of the feet,[42] so they do not train the tibialis anterior as much as the

quadriceps. This may explain the differences in muscle growth between the four muscles in infants with PWS and suggests that a training effect is strengthened by GH treatment.

We found that muscle thickness was correlated with muscle strength and motor performance in infants with PWS. However, while muscle thickness improved over time to the lower normal range, motor development remains seriously delayed in PWS infants.[9] This means that although decreased muscle thickness contributes to the motor performance problems, it can also be hypothesized that innate brain pathology affects motor and cognitive development.[43] PWS patients, in addition to the reported structural and functional muscle abnormalities[6,39,44] also show hypo-excitability of the cortical motor areas[45]. Studies of the PWS Necdin-deficient mouse model have reported a decreased number of motor neurons at birth[46,47] which is suggestive of innate structural abnormalities. However, the above mentioned constraints on muscles and neurological structures are also negatively influenced by disuse.

Although a sample of twenty-two infants with PWS seems small, this sample includes the majority of Dutch infants diagnosed with PWS diagnosed during inclusion period. The use of repeated measurements and MLRA increased the power of the study. We were able to achieve convergence and stable models with MLRA, although the number of cases on the second level is usually higher than the twenty-two infants in this study. Moreover, the models for all four muscle groups predicted the clinical observations well, reducing unexplained variance by 41-70%. We realize that a waiting list design is not optimal and differences in the control period are not ideal, however we think that our chosen methods minimized the disadvantages of this design.

Another problem was that the youngest PWS infants had not mastered the pulling task used to test muscle strength, leading to missing data, particularly during the control period. However, the significant, relatively high correlation between muscle thickness and both muscle strength and motor performance supported our hypothesis that GH improves muscle strength and motor development by increasing muscle mass in PWS infants.

Conclusion

GH has a positive effect on muscle thickness in PWS infants. Muscle thickness is highly correlated with muscle strength and motor performance. In muscles that are used a lot in the acquisition of fundamental skills in early motor development, there was a naturally occurring catch-up in growth independent of GH treatment, suggesting a training effect.

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7

General discussion

Introduction

This thesis focuses on motor development in infants with Prader-Willi syndrome (PWS) and the effect of child-specific physical training combined with growth hormone (GH) treatment on motor development.

In infants with PWS, feeding difficulties, a failure to thrive, severe muscular hypotonia, and muscle weakness result in serious motor developmental delay.[1-4] PWS infants are able to stabilize the head at 8 months of age in different positions, sit by themselves at 12 months, crawl at 19 months, and walk by 27-32 months of age.[5,6] It is obvious that this leads to a large care giving burden for parents when taking into account that in normal development, infants are able to stabilize the head at 3.5 months of age, sit at 8 months, crawl at 9 months, and walk at 13.5 months.[7] As a consequence of hypotonia and muscle weakness, PWS infants have major difficulties overcoming gravitational forces during rest, transport, and activities such as stabilizing and positioning the head and trunk and moving their limbs while reaching and grasping, rolling over, and sitting or standing upright. This has consequences for carrying the child: sometimes it is rather difficult to support these infants in a stable manner when picking them up from bed and during bathing, but also in the maxi-cosi while in the car, etc.

It is essential to stimulate motor development in PWS infants as early as possible, because motor activity is a vital factor in a child's ability to explore, interact, and learn from the environment. Each new motor skill creates new opportunities for perceptual, motor, and social exploration.[8]

However, at the start of this study, clinical studies focusing on early motor development in PWS were lacking and, as a result, only global insight into motor developmental patterns was present. Moreover, information concerning interventions to improve motor development at this young age were scarce. Therefore, we formulated five main research questions:

1. What is reported in the literature about motor problems in PWS, their underlying causes, and the effect of interventions on motor performance in PWS?
2. Which characteristics are typical in motor development for infants with PWS?
3. What is the effect of standardized child-specific physical training in combination with GH treatment on motor development?
4. Can muscle strength be objectively measured in normally developing infants and in infants with PWS?
5. What is the relationship between GH treatment, muscle mass, muscle strength, and motor development in PWS?

Question 1 is answered in chapters 2 and 3; questions 2, 3, 4, and 5 are addressed chapter 4, 5, and 6. Chapters 8 and 9 provide a short overview of the findings and results in English and Dutch, respectively. In this chapter, gaps, new ideas, and findings in clinical thinking, practices, and research on motor problems in PWS are discussed using the International Classification of Functioning, Disability and Health Children and Youth version (ICF-CY) of the World Health

Organization [2008]. This classification model is useful to organize information on health conditions and on interacting factors. Firstly, motor problems in PWS are described according to the ICF-CY level of body structures and functions, followed by an approach on the ICF-CY level of activity and participation. Secondly, a sketch of our randomized controlled trial (RCT) and a reflection on the design and technical considerations are presented, followed by a discussion about the challenges in organization and implementation of infant-centered pediatric physical therapy care for rare diseases such as PWS. After this, we discuss an objective new measurement method to assess muscle strength in infants, which was developed to test our hypothesis on the relationship between muscle mass, muscle strength, and motor development in PWS infants. Then the findings of the RCT concerning the effect of training and GH on motor development in PWS infants in relation to the possible underlying causes of motor problems in PWS are discussed. Finally, the results of our studies, the implications of these findings, and recommendations for the scientific field and clinical practice are presented.

Dominant perspective in literature: Body functions and structures cause motor problems in PWS

The dominant thought in literature is that hypotonia, muscle weakness, and motor developmental delay in PWS patients are related to their abnormal body composition with increased fat mass and decreased lean body mass (LBM), which mainly consist of muscle tissue (Figure 1, oval). Because body composition in PWS resembles that of GH deficient patients and because those with PWS have hypothalamic abnormalities,[9] GH deficiency is typically suspected. This was tested and, indeed, GH secretion is decreased for those with PWS.[10] From the 1990s onward, the effect of GH treatment in PWS patients was evaluated and various studies showed that GH treatment positively influences body composition.[11-16] It was reasoned that GH will increase muscle strength, first by increasing muscle mass and second by decreasing fat mass, which will result in relatively more muscle strength and therefore better motor development.[11,17,18] Following this reasoning, researchers evaluated whether GH treatment improved motor development in PWS infants. Indeed, GH yielded positive effects on motor development[4,12,18] and on agility and thoracic muscle strength in PWS children.[13,19,20] However, most studies focused on descriptive changes in the muscle-fat ratio and changes in motor performance as result of GH treatment without strong evidence for a *causal relationship*. The ICF-CY allows us to describe all findings reported in literature through a classification model which shows that some findings are neglected in the reasoning model (Figure 1, upper panel). Research to further understand the underlying causes of the muscle abnormalities and the relationship to motor problems in PWS is scarce. Moreover, a description of motor problems at the activity and participation level is lacking, while this is needed to design interventions which fit to daily life and individual needs of PWS patients in infancy, childhood, and adulthood, as well as their parents.

	Body functions	Body structures	Activity & participation	
Literature	- Hypotonia - Muscle weakness - Endocrine function	- Body composition: fat mass↑ muscle mass↓ - Abnormalities hypothalamus	- Delayed motor development - Decreased activity	
	- Hypermobility - Hyperlaxity - Hypo-excitability motor cortex	- Type-1 muscle fiber size↓ - Type 2 muscle atrophy - Abnormalities muscle fiber: contractile elements, mitochondria	- Balance control↓ - Abnormal gait pattern - Decreased fitness	**In infants** - Language abilities↓ - Feeding difficulties
Results RCT	**In PWS infants** - Muscle strength is about 40% decreased	**In PWS infants** - Muscle thickness is decreased (about -1.5 SDS)	**In PWS infants** - Motor development varies significantly - On average, it takes twice as long to reach motor developmental milestones - Infants with more motor delay early in life remain more delayed over time than infants with relatively less delay. - Over time motor developmental rate decreases - Walking at 27 (SD = 5.8) months of age. - At about 42 months of age, most infants are able to walk and run, but are not able to jump or climb the stairs	- Decreased ability to interact with and explore the world around them - Difficulties in handling and caretaking - Difficulties in transporting the child - Special attention for child's seat, car seat, carrycot - Special attention for the fitting of shoes - Need for supporting materials, such as support cushions
	- Physical training combined with GH has a positive clinical relevant effect on motor development. - Muscle thickness is highly correlated with the decreases in muscle strength and motor performance. - Physical training combined with GH increases muscle thickness matched by increases in muscle strength and motor development.			

Figure 1. Motor performance problems in PWS organized among the description levels of the International Classification of Function, Health, and Disability (ICF-CY) of the World Health Organization
The findings reported in the literature are presented in the upper panel and the findings of this thesis are presented in the lower panel. The focus in clinical thinking, practice, and research is mainly on items at the level of body functions and structures (oval) our more problem based approach provided new information on all three levels and the on the relationship between them (rectangle).

A problem based approach to motor development in PWS infants

In our opinion, it is evident that because of the severe hypotonia and decreased muscle strength in PWS infants[1,2] together with their abnormal body composition,[11,12,18,21] their body is not automatically attuned to move in its environment. This is seen by the fact that PWS infants move

less frequently[1,22,23] and are especially less able to perform antigravity movements such as holding and moving the head, trunk, and limbs in different positions.[1,23] From the clinical practice, it is known that PWS infants move less and much slower than normally developing children and get easily fatigued, which leads to a marginal amount of movement repetitions that constrains skill acquisition considerably. Thelen (1984) demonstrated that in developmentally normal infants, body composition is a critical factor to overcome gravitational force. Newborn infants make kicking movements in the supine position and step movements, when held upright, which are kinematically similar. Although the kicking movements remain, the step movements disappear within the first few weeks of life. She explained this phenomena by the fact that infants acquire fat at a greater rate than muscle mass leading to an imbalance between fat and muscle mass and relatively decreased muscle strength, which results in an inability to overcome gravity forces in the upright position.[24,25] When muscle strength increases as result of training and growth, the stepping movements reemerge again. Likewise, young infants are more able to reach from a supported half-sitting position compared to reaching from a supine position, because more force is needed in supine position to initiate a reaching movement then in a half-sitting position[26]. By manipulating body orientation in such a way that gravity has less influence on the body, we hypothesized that it would be possible to improve the motor learning conditions for PWS infants and enable them to practice and learn new skills. Moreover, we concluded that these infants need adequate support during the phase that muscle strength is too low to adequately control posture in different positions.

Based on previous literature (Chapter 3), it could be concluded that physical training without GH has beneficial effects in PWS children and adults in terms of body weight, body composition, activity level, physical fitness, or a combination of these.[27-33] Therefore, it was obvious that physical training is effective in PWS patients and should be integrated in the clinical management of PWS, especially in young infants. Moreover, it is obvious that in such training the influence of gravity has to be taken into account to increase both muscle strength and skill learning.

The challenges in the randomized controlled trial (RCT).

Taking into account the relationship between body structures and functions in relation to activity and participation levels, we reasoned that GH might be more useful to improve muscle strength and skill learning if the infant is also stimulated to actually practice motor skills. Therefore, we hypothesized that GH treatment would increase the effect of physical training in PWS infants. Moreover, it was obvious that there was the question for support among parents, who needed to take care for their infant in a way adapted from developmentally normal infants. Such a program should be home-based. Therefore, we developed a child-specific physical training program in which the parents and local pediatric physical therapists were coached by two specialized pediatric physical therapists from the Radboud university medical center (Isabel Durein, Annelot Zweers). The program focused on adequate support as long as needed and both the stimulation of muscle strength training and motor skill learning among PWS infants. The general principle

was to manipulate the learning environment in such a way that the child was able to handle gravity, to train muscle strength, and to learn motor skills by repeated practice attuned to the needs and developmental level of the infant (Chapter 4). This intervention was based on the principles of Esther Thelen and the Repetition Maximum (RM)-model,[34, 35] and motor learning theories.[36] To test effectiveness, we started a longitudinal randomized clinical trial (RCT) of two years in PWS infants in which all infants participated the training program from the start of the study and the start of GH treatment was randomized.

It was a challenge to find the most adequate design for our study. The best design would have been a RCT with four groups: no specific intervention but usual care, infant centered physical training, GH treatment without training, and GH treatment with training. However, due to the low incidence of PWS, this would have taken many years of research with changing circumstances. Besides, it would have been impossible and unethical to omit the training program with parental coaching. Therefore, we chose a design in which all infants participated in the training program from the start of the study, and GH treatment was randomized. However, the control group received GH treatment after a relatively short control period, because although the study started with a control period of one year, it was shortened to six months during the study because the results of Festen and colleagues (2008)[4] demonstrated effectiveness of GH on motor development. To increase statistical power we measured the infants every three months.

The objective of our study was first to evaluate whether the combined intervention of GH treatment and training improved motor development and next to describe motor development in PWS infants in detail. To best answer these questions, we needed to study developmental change over time.[37] By investigating motor performance longitudinally, one obtains information about the way motor test outcomes rise and fall over time within each infant (within-individual change). Based on this information, it is possible to assess whether different infants manifest different patterns of change and likewise to detect factors that predict these differences. In other words, longitudinal data can provide answers to questions of systematic individual differences in change as a consequence of training combined with GH treatment. For such data, so-called multilevel growth models have been developed.[37] These models assume that all individual change trajectories have a common algebraic form from which each individual growth trajectory can be derived[37]. The algebraic form of one of the motor tests (AIMS) was a linear function of age (Figure 2d of Chapter 4). However, the rate at which each infant changed over time varied significantly between infants. This was partly explained by GH treatment and by baseline motor developmental level. GH treatment increased motor developmental rate but PWS infants with an initially lower motor level remained more decreased over time compared to PWS infants with initially higher motor developmental levels (Chapter 4).

The chosen design and analysis method had several advantages. First, by measuring motor performance repeatedly, the statistical power of the study increased although the sample (22 infants) was quite small because of the low incidence of PWS. Second, the relatively large age range and the differences in length of control period, both due to performing our research in a

clinical setting, were compensated for as multilevel analyses allow data in which the timing and spacing of the repeated measures differ across people.[37] Third, the design and analysis method coped with both inter-individual differences between PWS infants and intra-individual differences within PWS patients. Therefore, although not common in clinical research, a longitudinal design combined with multilevel analysis provides a powerful instrument to analyze data related to developmental processes.

Challenges in clinical care: transmural organization of child-specific care in PWS

In the Netherlands, it is typical that all PWS infants receive regular pediatric physical therapy, first at home and later in a center nearby home. Because the incidence of PWS is very low, pediatric physical therapists do not have experience with or specific knowledge about the syndrome. In an expert center, such as the Radboud university medical center, a concentration of PWS patients and the availability of a multi-disciplinary team allow a better understanding of patients' special needs. When the center, the family's physical therapist, and the parents work together, an exchange of knowledge and experience can be achieved.

In this study, the expert center saw PWS infants every three months (with alternating hospital and home visits). The pediatric physical therapist was contacted, information about the syndrome was provided, and the principles of the intervention were discussed. Moreover, we discussed how to exchange information and the family's physical therapist was invited to be present during home visits made by the Radboud team. During the visits, questions from parents and pediatric physical therapists were discussed, motor performance was evaluated, training goals were set, and exercises and environmental adaptations were discussed. The test results, answers on questions, recommendations, and proposed exercises were given by oral and written reports by e-mail and a specially designed website. This website contained general information about PWS and included a secure, separate personal section for each infant in the study. Between visits, the parents and the physical therapist were invited to contact the center when there were questions. Besides coaching the parents and the physical therapist in muscle strength training and stimulating motor skill learning, the Radboud team provided information about typical problems in PWS infancy. For example, this information included feeding possibilities, a balance between activity and rest, safe sleeping positions, postural support during transport, handling instructions, etc. Moreover, information about supportive materials was given (Chapters 1 and 4).

Satisfaction with this program was evaluated using a parent questionnaire and a physical therapist questionnaire (Table 1, unpublished data). For each domain of the program, questions were asked on a four-point scale and, for each domain, the survey allowed individual remarks. Table 1 presents the mean results per domain. For example, 19% of the questions regarding the information about the research project and measurement procedures were scored as very positive (Table 1).

Table 1. Outcome of the parent questionnaires per domain after one and two years of intervention and outcome of the pediatric physical therapy questionnaire

	Parents One year (%)				Parents Two years (%)				Pediatric physical therapists (%)			
Domains	**1**	**2**	**3**	**4**	**1**	**2**	**3**	**4**	**1**	**2**	**3**	**4**
Information about the research project and measurement procedures	19	75	6	0	21	78	0	0	32	68	0	0
Evaluation of motor performance	55	43	2	0	62	38	0	0	Not questioned			
The reports on motor performance	36	59	5	0	37	62	1	0	40	57	3	0
Exercises and recommendations	26	58	14	0	40	52	8	0	40	51	9	0
Collaboration	Not questioned				Not questioned				24	72	4	2
Transmural care	30	56	12	3	33	41	24	3	10	59	30	1
Project team	66	34	0	0	70	30	0	0	43	57	0	0
Organization of the project	63	35	0	2	72	28	0	0	Not questioned			

1=very positive, 2=positive, 3=negative, 4 very negative

Parents' answers after the first and second year of the study were very similar, but slightly more positive after the second year. Overall, the parents were positive about the program, but the answers about the exercises and recommendations and transmural care showed more variation and also some negative evaluations. Parents remarked that they valued exercises and practical recommendations, but some parents reported difficulty in translating the exercises to the at-home setting or with respect to the continuously developing infant. If this was the case, usually the pediatric physical therapist of the family helped the parents to attune exercises to a specific circumstance. In evaluating the transmural care, parents reported finding it difficult to compare the transmural care to usual care because they did not experience how the care would have been without the expert center. However, the parents remarked feeling supported by the transmural organization and highly appreciated exchanging experiences with the expert center and to receive advice specific to PWS.

Pediatric physical therapists were positive about the program as well, but slightly less optimistic compared to the parents. They scored the exercises and recommendations as well suited, but the answers about the transmural care showed more variation. Some therapists valued the program and remarked that the recommendations, exercises, and the underlying training principles were innovative and helped them in clinical practice. However, 30% of therapists reported that recommendations were in agreement with their usual practice; most of these therapists appreciated that their practice was confirmed and felt supported, and some therapists judged that the expert coaching had minor additive value.

The way the pediatric physical care was organized during this study was assimilated with a lower frequency of visits (six monthly instead of three monthly) in the Dutch protocol for diagnosis and management of PWS (http://www.nvavg.nl/upload/diverse-publicaties/2013--stichting-kind-en-groei-diagnostiek-en-behandeling-van-kinderen-met-het-pws.pdf).

Muscle strength measurement in infants

The aim of the RCT was to attain insight into the relation between muscle strength, muscle mass, and motor performance in PWS infants and to evaluate the effects of child-specific physical training with or without GH over time. Therefore, there was an objective need to measure muscle strength in PWS infants. This was a challenge because the regular measurement techniques for muscle strength are not suited for infants. Clinicians determine muscle weakness in infants subjectively by observing the possibility of antigravity movements and by testing muscle strength manually, using several maneuvers such as the so-called 'pulling to sit', 'scarf sign', 'shoulder suspension', and 'ventral suspension' methods.[38,39] However, on the basis of these observations, it is possible to discriminate between normal muscle strength and the presence of hypotonia and/or decreased muscle strength. It is not possible to determine to what extent muscle strength is diminished, which hinders monitoring changes over time and does not allow comparison to normal development. Moreover, it is also difficult to determine whether the infant suffers from both hypotonia and decreased muscle strength or from hypotonia only,[38] which is important in diagnosing a hypotonic infant,[40,41] because hypotonia, combined with severe muscle weakness, indicates a peripheral origin so that when muscle strength is only mildly effected, a central origin is more likely.[38,40,41]

Because objective measurement methods were lacking and little knowledge was available about muscle strength in infants in general, and specifically with regard to infants with motor problems, we developed a new measurement instrument to quantify muscle strength in infants and toddlers: the "Infant Muscle Strength meter" (IMS-meter). In the developmental process, we discussed several ideas with human movement scientists, pediatric physical therapists, and technicians. Although normal muscle strength measurements are based on resistance measurements, we decided to use pulling actions because, in young infants aged 6 months and older, pulling is natural behavior to get a desirable object while pushing behavior occurs in later development. Comparable to the existing muscle strength measurements, we tried to evoke a maximal pulling action and developed a measurement instrument and method to register and identify pulling activities between other movements related to changing positions (Chapter 5). This was necessary because the registration was based on a strength sensor under the chair. We were able to determine muscle strength in young infants between six and 36 months of age in a reliable, objective, and reproducible way and developed a prediction model for normal muscle strength based on reference data among normally developing infants. Using the IMS-meter and the prediction model, low muscle strength as a determining factor of motor developmental problems in infants with PWS could be established (Chapter 5 and 6). The IMS-meter has the potential to improve clinical practice for infants with muscle weakness from about six months of age.

Motor development in PWS infants: new findings from the RCT

Although motor development can vary widely among infants with PWS, these patients have a specific pattern of development. In the RCT, the infants took approximately twice as long to reach motor development milestones as infants who develop typically; in addition, over time, motor developmental rate decreased and the infants seemed to reach, for that moment in time, their maximal potential, which was highly different from normal development (Figure 1, rectangle). So, although the combination of GH and training resulted in a significant clinically relevant improvement in motor development, it does not normalize it (Chapter 4). Results regarding muscle thickness pointed to the effectiveness of physical training independent from GH. In the forearm flexor and quadriceps, muscle thickness caught up before the start of GH, which was nevertheless absent in the biceps brachii and tibialis anterior. In chapter 6, we presumed that this was related to the type of learned movements in typical early development. This means that some muscles are more involved in these early movements than others, for example, in manipulating objects in a pronation position of the lower arm, forearm flexors are addressed more than the biceps brachii. The quadriceps is especially addressed when the infants learn to stand up and walk while the tibialis anterior is less addressed during these activities, because early walkers do not use dorsal flexion of the feet[42] (Chapter 6). These findings contributed toward a better understanding of motor development in PWS infants and, although these findings gave insight in the influence of GH on muscle mass, muscle strength and motor development, the long-term effect on development is not clear yet.

Until now, motor problems in PWS are presumed to be mainly related to an abnormally high fat: muscle ratio. In PWS, the fat: muscle ratio increases over time, as the percentage of body fat increases, while muscle mass does not increase accordingly (Chapter 2). Because of the rough linear relationship between muscle strength and muscle cross-sectional area,[43] we reasoned that muscle strength should be decreased in PWS patients by about 25-37% based on the measurements of muscle mass (Chapter 2). The findings in the RCT, that muscle thickness is decreased in PWS infants and related to muscle strength and motor performance (Chapter 6), add knowledge to the existing literature (Figure 1, rectangle).

However, there are some findings that do not fit the explanation that motor problems in PWS infants are mainly caused by abnormal body composition. Firstly, body composition is less affected in PWS infancy compared to childhood and adulthood, whereas motor problems are most severe in infancy (Chapter 2). Secondly, in the RCT, it was found that muscle thickness improved over time to the lower normal range (Chapter 6), but motor developmental delay remained severe (Chapter 4). Thirdly, the muscle strength of PWS infants was on average 60% of normal reference (Chapters 5, 6), which is in line with the findings of decreased muscle mass. However, the results of the muscle strength study (Chapter 5) indicated that among 24 month-old PWS infants, some infants produced normal muscle strength. Moreover, unpublished data from the longitudinal muscle strength measurements in PWS infants of the RCT indicated that not all PWS infants demonstrated decreased muscle strength at all points in time. There was

even one PWS infant that produced normal muscle strength throughout the study although his motor development was severely delayed. Although muscle thickness, muscle strength, and motor performance are related for PWS infants (Chapter 6), muscle thickness is not an unequivocal predictor for strength and motor performance among them.

There are also other factors that may contribute to motor problems in PWS. First, structural and functional muscle abnormalities[1,44,45] and the hypo-excitability of the cortical motor areas[46] are reported. These findings can both be the result of innate cortical and/or muscle pathology or of disuse (Chapter 2). However, out of the scope of this thesis, we did in collaboration with the Laboratory of Animal Science, Radboud university medical center (Carlijn Hooijmans and Merel Ritskes-Hoitinga), a broad systematic literature search focused on PWS animal models. The search was focused on motor performance and neuromuscular abnormalities. Only two studies were found on this topic. The most prominent outcome was that, in PWS Necdin-deficient mouse, increased natural occurring cell death during embryonic development led to a 31% decrease of lumbar motor neurons at birth.[47,48] Authors reported that the lack of Necdin is involved in motor deficiency in PWS patients,[47,48] which indicates innate structural abnormalities in the neurological system which are possibly related to the hypo-excitability of the cortical motor areas. Moreover, a lower number of motor neurons means also a lower innervation level of the muscles with consequences for muscle strength and coordination. Therefore, we conclude that although body composition is an important contributing factor to the motor problems in PWS infancy, abnormalities of the central nervous system may contribute to the clinical picture as well.

Furthermore, initial motor performance levels influenced motor development over time. Infants with a larger motor delay early in life continued to have more motor delay over time compared to infants with relatively less severe motor delay (Figure 1, rectangle). Because this might be related to the genetic variance in PWS, it would be interesting to evaluate motor developmental differences in the PWS genetic subtypes. However, because of the low incidence of the syndrome, our study sample was too small to do such evaluations.

Implications of this thesis for the scientific field and the clinical practice

Implications for the scientific field

This thesis revealed detailed insight into the motor developmental pattern of PWS infancy and a significant and clinically-relevant effect of child-specific physical training, combined with GH treatment, on muscle thickness and motor development. The RCT ended before the infants were able to jump or climb stairs using an alternating step pattern. From previous literature, we know that in childhood and adulthood motor problems remain (Chapter 3) but knowledge on the maximum level of motor performance in comparison to typically developing peers is not clear yet. Long-term longitudinal research is needed to determine if —and at what age— final motor milestones are reached and to evaluate the long-term effects of early intervention of GH and training on general development. Research in patient groups with low incidence, as in PWS, are

difficult to perform especially in children where you need to control for developmental changes. We recommend longitudinal multicenter designs with an adequate number of repeated measures in each individual patient to increase the statistical power and to study changes over time (within-individual change) and inter-individual differences in change. Moreover, the underlying causes of motor problems in PWS should be studied more extensively. Based on the results of this thesis, the body composition abnormalities cannot solely explain the severe motor problems in PWS infancy. Findings of PWS Necdin-deficient mouse with a decreased number of motor neurons at birth,[47,48] the hypo-excitability of cortical motor areas in PWS adults,[46] and a possible relationship between brain abnormalities and hypotonia[49] point into the direction of innate central nervous system defects. In the same manner as endocrine abnormalities in PWS are related to defects of the hypothalamus,[50-52] motor problems could be related to central nervous system defects.

Implications for clinical practice

Based on our results, we recommend physical training combined with GH treatment in PWS infancy, taking into account muscle strength and skill learning in relation to growth and development. It is really important to offer the child even in the hypotonic phase enough additional support to play in a meaningful manner and to combine exercises with learning environments. It has to be taken into account that the child needs to be able to control its posture with or without supporting materials to avoid scoliosis in an early stage. Scoliosis, defined as a Cobb angle of more than 10˚, has an overall prevalence in PWS in between 30-80% of patients.[53,54] The prevalence and severity of scoliosis increases with age,[53,55] but already in infancy about 20-30% of PWS patients have scoliosis.[53,54] The pathogenesis of scoliosis is not yet clear, but it is hypothesized that it is related to hypotonia and muscle weakness in PWS.[55] There are two types of scoliosis in PWS, a long C-curve, especially present in young PWS infants, and a S-scoliosis which is comparable with the idiopatic adolescence scoliosis but develops at an earlier age in PWS children. S-scoliosis is especially progressive into adult age. Until now, no negative effect of GH treatment is found on the development or progression of scoliosis.[53,55]

So during the intervention, it is a challenge to search for a balance between support to handle gravity and activate motor development to open up new opportunities for PWS infants to interact and learn from the environment. Since brain development is guided by an ongoing interaction between the organism and the environment[56] and, in the first two years of life, major brain developmental processes take place,[57-59] motor development is a strong stimulator for structural and functional brain development in infancy.[8,60,61] Therefore, stimulating motor development in young PWS infants may improve later developmental outcomes.

To increase muscle strength and improve motor skills, physical training should be an integrated aspect of daily life. Therefore, interventions for PWS infants should be focused on empowering parents about how to support their infant by providing a playful learning environment attuned to their child's constrained motor abilities. Only by coaching the parents are infants able to practice

daily. Moreover, after infancy, we recommend that physical activity and muscle strength training remain an important part in daily life in PWS patients to manage weight control during the phase of hyperphagia. This means that being active should be an enjoyable natural part of life. People with PWS are at risk for a vicious circle in which increased weight increases problems that affect gross motor skills, which has a negative influence on activity level and will negatively influence muscle strength and physical fitness. These results, of course, entail a more sedentary lifestyle with all of its associated health problems.

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Summary

This thesis describes a number of studies performed to increase understanding of motor problems in Prader-Willy syndrome (PWS), its underlying causes, and interventions to improve the motor developmental process in PWS infants.

PWS is a rare genetic multisystem disorder characterized by hypotonia, hyperphagia, obesity, short stature, mild dysmorphic facial features, cognitive and behavioral deficits, and endocrine disturbances like hypogonadism and GH deficiency. In infancy, feeding difficulties, failure to thrive, severe muscular hypotonia, and muscle weakness result in serious developmental delay. Because of the wide variety of systems affected, PWS patients need specialized multidisciplinary care. However, the parents also require specialized guidance and support in taking care of their child. Because the syndrome has a low incidence, healthcare professionals are usually not familiar with the syndrome. In The Netherlands, about ten infants with PWS are born each year. Therefore, specific PWS expertise is concentrated in a few centers. One of them is the Radboud university medical center, at which the need for a more evidence based approach in the care of PWS infants originated, which in turn motivated initiating this thesis.
The aim of this thesis was threefold: first, to contribute towards a better understanding of motor development in infants with PWS; second, to gain insight into the effect of child-specific physical training combined with GH treatment on muscle strength, muscle mass, and motor development; and, third, to increase the quality of care for these infants and their parents by developing a child-specific intervention program.

Five main research questions were defined:
1. What is reported in the literature about motor problems in PWS, their underlying causes, and the effect of interventions on motor performance in PWS?
2. Which characteristics are typical in motor development for infants with PWS?
3. What is the effect of standardized child-specific physical training in combination with GH treatment on motor development?
4. Can muscle strength be objectively measured in normally developing infants and in infants with PWS?
5. What is the relationship between GH treatment, muscle mass, muscle strength, and motor development in PWS?

Chapter 1

Introduction

Although motor problems in infancy are distinctive characteristics of PWS and of great concern for parents, clinical studies focusing on early motor development in PWS are lacking. As a result, insight into the motor development pattern in these infants is limited and information concerning interventions to improve their motor development is scarce. Therefore, this thesis focuses on motor development in infants with PWS and the effect of child-specific physical training combined with growth hormone (GH) treatment on motor development.

PWS is characterized by a wide variety of physical, cognitive, and behavioral defects and results from lack of expression of a paternally imprinted region of chromosome 15q11-13. Many of the symptoms in PWS are thought to be the result of hypothalamic dysfunction. In PWS, several neurological abnormalities in the structure and function of the hypothalamus have been reported. Moreover, the hypothalamus is involved in regulating the endocrine and autonomic nervous systems, both of which are affected in individuals with PWS. In infancy, muscular hypotonia, poor sucking reflexes, feeding problems, failure to thrive, and psychomotor delays are most prominent. In childhood, hyperphagia as well as cognitive, social, and behavioral deficits become more prominent. In adulthood, there is a lack of complete pubertal development and cognitive, emotional, and psychiatric problems are common.

The child-specific physical training used in this thesis was based on a dynamic systems perspective on (motor) development. In this perspective, developmental changes are the result of a complex interaction between the child, its environment, and goal-directed actions or tasks. Likewise, the nervous system is shaped by experience and interaction with the environment. Studies on newborn stepping (stepping reflex) demonstrated that task performance (stepping) was influenced by the existing interaction between growth and changing body characteristics and the environment, especially in relation to gravity. From this perspective, hypotonia and low muscle strength in PWS infants are important constraints in overcoming gravity and repeating movements with sufficient frequency to achieve a learning effect. Interventions should be focused on manipulating the task and environment in such a way that the child gains the ability to explore its biomechanical properties and simultaneously learns how to use its constrained motor abilities in a functional manner.

The motor developmental problems of PWS infants encountered in daily life, the challenges for their parents in caregiving, and some elements from the intervention program are illustrated in a clinical case report (Chapter 1, boxes 1-4).

Chapters 2 and 3

An extensive literature search in four databases (1956-2010/2011) was performed to provide an overview of existing knowledge about motor problems in PWS, the underlying causes, and intervention to improve motor performance in PWS. The results were reported in two systematic

reviews, the first concerning the presumed underlying causes of motor problems in PWS and the second concerning motor problems and interventions to improve motor performance.

Chapter 2

Motor problems in PWS are presumed to be related to abnormal body composition, with increased fat mass and decreased lean body mass (LBM) and possibly some neuromuscular abnormalities. The systematic literature search revealed 38 references that met the inclusion criteria. Nine studies focused on body composition, seven on neuromuscular functioning, and 22 on the effect of GH treatment on body composition. All the body composition studies reported increased fat mass and decreased LBM in PWS. Body composition is already somewhat out of balance in infancy, but this worsens in childhood and adulthood. In PWS infants, levels of 28 to 32% were reported, while normally infants have about 24% body fat. In PWS children, levels of 36 to 55% were reported, while at this age body fat is normally about 18%. In PWS children LBM, which mostly consists of muscle mass, was found to be 50 to 60% of total body mass, whereas in normal children this is about 80%. From these findings, we determined that muscle mass is 25 to 37% decreased, which might explain the weakness and hypotonia in PWS. However, since motor problems are most severe in infancy but body composition at this young age is otherwise only mildly effected, neuromuscular abnormalities may contribute to the motor problems as well. There were only a few studies focusing on this subject and they reported both structural and functional muscle abnormalities as well as hypo-excitable cortical motor areas in PWS, but it is unclear whether these abnormalities are the result of innate pathology, a secondary phenomenon of disuse, or a combination of both.

All of the GH studies reported positive effects of treatment on body composition. GH decreased the percentage of body fat and increased LBM. However, because GH also increases growth, LBM should be corrected for height. Studies in which this was done reported that, in PWS children, LBM corrected for height normally decreases, but under the influence of GH it stabilized. However, in PWS infants, GH did increase LBM corrected for height. Because physical training also improved body composition in PWS patients and might prevent a decrease in muscle mass as a result of disuse, we suggest that physical training combined with GH could enhance each another.

Chapter 3

Although motor problems in PWS are prominent, there is little insight into the factors relevant for clinical management. This study was performed to provide an overview regarding the characteristics and prevalence of motor problems in PWS and to summarize the reported effects of GH treatment and physical training on motor performance. The systematic literature search revealed 34 references: 13 on motor performance, 12 on effects of GH treatment on motor performance, and nine on physical training in PWS patients.

In PWS infants, motor development is 30–57% of the normal reference values. In PWS children and adults, activity level, physical fitness, and muscle strength are decreased. Also, in PWS adults, decreased balance capacity and abnormal gait patterns are reported. Based on these findings, we concluded that motor performance in PWS infants should be studied longitudinally to evaluate the consequences over time. Moreover, motor deficiency in PWS adults should be studied in relation to structural and functional impairments connected to the consequences for participation in daily life.

Most studies, but not all, reported positive effects of GH treatment on motor development in PWS infants. In PWS children and adults, positive effects of GH on motor performance were also reported. Studies on physical training in PWS reported beneficial effects in children and adults with regard to body weight, body composition, activity level, physical fitness, or a combination of these. We concluded that physical activity and muscle force training need to be an important part of daily life in PWS patients of all ages and suggested a combination of GH treatment and physical training started as early as possible, especially in infants.

Chapter 4

Severe hypotonia, muscle weakness, and motor developmental delay are characteristic of infants with PWS. Interventions to improve motor development have focused on GH treatment, but the results are contradictory. Although physical training is beneficial in PWS children and adults there are no reports about its effect in PWS infants. The motor development pattern over time has never been studied in PWS infants, and the effect of GH on motor performance has never been evaluated in relation to the developmental pattern. Therefore, we studied whether GH treatment could enhance the effect of physical training on motor development in PWS infants longitudinally. Twenty-two infants were followed for two years in a randomized controlled trial (RCT). Both groups followed a child-specific physical training program from the start of the study. The treatment group started GH after baseline measurements and the control group started GH after a control period. Motor performance was measured every three months using three standardized motor tests: the Alberta Infant Motor Scale (AIMS); the Motor Scale of the Bayley Scales of Infant Development, 2nd edition (BSID-II); and the Gross Motor Function Measure (GMFM).

We found that, on average, it takes PWS infants approximately twice as long to reach motor development milestones compared to infants with normal development and, over time, the rate of motor development decreases and appears to plateau. Multilevel regression analysis revealed that the motor developmental patterns differed significantly between infants. This was partially explained by the baseline level of motor development and the effect of GH treatment. First, infants presenting initially with a greater motor developmental delay remained more delayed compared to infants presenting initially with relatively less motor delay. Second, GH treatment had a positive and clinically relevant effect on motor development. The AIMS multilevel model indicated that infants treated with GH reached the end of the test 3.8 months sooner, on

average, than without GH treatment. Moreover, the GMFM multilevel model indicated that infants treated with GH are able to run, step over a stick at knee-height, and pull to a sitting position without head lag; however, without GH treatment the infants are only able to walk independently and stand on one leg for a few seconds, but are unable to run and have a head lag when pulling to a sitting position. Furthermore, we found indications that early treatment with GH results in an earlier increase in the rate of motor development and a higher final level of development.

In conclusion, GH treatment enhanced the effects of child-specific physical training but, despite this positive effect, a major delay in motor development remained. Moreover, the correlation between the age at which GH treatment was started and the age at which the infants achieved a maximal motor score indicated that the effect was more pronounced when GH treatment was initiated at a younger age.

Chapter 5

Evaluation of muscle strength in infants is difficult since no objective measurement methods are available. The clinical evaluation of an infant with motor development delay, muscle weakness, and hypotonia would improve considerably if muscle strength could be measured objectively, because hypotonia combined with severe muscle weakness indicates a peripheral origin, and hypotonia combined with relatively mild effected muscle strength indicates a central origin. If muscle strength could be quantified, it would be possible to evaluate interventions to improve muscle strength. Therefore, we developed a method to measure muscle strength in infants, the *infant muscle strength meter* (IMS-meter) using a pulling task. The pulling task was based on naturally occurring pulling behavior, which infants aged six months and older perform when they try to obtain a desirable object. The aim of this study was to determine whether maximum muscle strength during such a spontaneous reaction in normally developing infants and infants with PWS can be measured reliably, to gather reference data and develop a model to predict muscle strength.

Eighty-one normally developing infants, 6–36 months of age, and 17 PWS infants 24 months of age were included. In a convenient subgroup of 46 normally developing infants, IMS was measured twice by two different assessors to establish the inter-rater reliability of the IMS-meter, which was found to be good. Moreover, the convergent validity of the IMS-meter was confirmed by the strong correlations between muscle strength, age, height, weight, and motor performance. A multiple linear regression model was developed to predict muscle strength based on age, height, and weight, explaining 73% of the variance in muscle strength in normally developing infants. In PWS infants, muscle strength was significantly decreased, but the variation was also high and three infants scored with normal muscle strength. Muscle strength was found to be a restrictive factor on motor development in PWS infants, because infants in whom muscle strength was more severely affected also had a larger delay in motor development. However, more IMS reference data are needed to optimize the IMS prediction model. Furthermore, the

IMS meter needs to be further explored in other patient groups between 6 and 36 months of age and technical improvements are needed for use in clinical practice.

Chapter 6

GH treatment has a positive effect on body composition and motor performance in PWS. Although it is hypothesized that GH treatment improves muscle mass and thereby muscle strength and motor development in PWS infants, studies that demonstrate this relationship are lacking. Moreover, physical training also has a positive effect on body composition and motor performance in PWS. Therefore, this study investigated longitudinally the effect of child-specific physical therapy combined with GH treatment on muscle thickness and its relationship with muscle strength and motor performance in PWS infants.

In a RCT, twenty-two PWS infants were followed over two years comparing a treatment and a waiting list control group. Using ultrasound, the muscle thickness of four muscle groups was measured: the left biceps brachii, right forearm flexors, right quadriceps, and left tibialis anterior muscle. Muscle strength was evaluated using the Infant Muscle Strength meter (IMF-meter). Motor performance was measured with the Gross Motor Function Measurement (GMFM). It was found that muscle thickness was significantly decreased in all four muscle groups, while muscle structure was normal as measured by muscle echo intensity. GH treatment combined with physical training significantly increased muscle thickness into the lower normal range. Catch-up growth was faster in the muscles that are used most frequently in early development. As this effect was independent of GH, it indicates a training effect. Moreover, it was demonstrated for the first time that in PWS infants, decreased muscle thickness is strongly associated with decreased muscle strength and motor performance. However, while muscle thickness improved over time into the lower normal range, motor development remained seriously delayed in PWS infants.

Chapter 7

We used the International Classification of Functioning, Disability and Health Children and Youth version (ICF-CY) of the World Health Organization [2008] to organize the findings reported in the literature about motor performance problems in PWS and the underlying causes of these problems. This revealed that the focus was mainly on items at the level of body functions and body structures and that some findings were neglected.

It is evident that, because of the severe hypotonia, decreased muscle strength and abnormal body composition in PWS infants, their bodies are not automatically attuned to move in their environment. Therefore, we approached the motor problems on the ICF-CY level of activities and participation. In normally developing infants, body composition is a critical factor in overcoming gravitational force. Experiments from a dynamic systems perspective on motor development revealed that by manipulating body orientation in such a way that gravity has less influence on the body, infants were better able to perform particular motor tasks. We hypothesized that it

would be possible to improve the motor learning conditions for PWS infants in the same manner, which should enable them to practice skills and learn new skills.

We reasoned that GH might be more useful for improving muscle strength and skill learning if the infant was also stimulated to actually practice motor skills. Therefore, a child-specific physical training program was developed in which the pediatric physical therapy care was organized transmurally. The parents and the pediatric physical therapist of the family were coached by therapists from the PWS expert center. To test the effectiveness of training combined with GH we performed a longitudinal RCT for two years in PWS infants. All the infants participated the training program from the start of the study. The treatment group started GH immediately after a baseline measurement, while the control group started GH after a control period. By investigating motor performance longitudinally, we obtained information about the way motor test outcomes rose and fell over time within each infant (within-individual change). Based on this information it is possible to assess whether different infants manifest different patterns of change, and detect factors that predict these differences, using multilevel analyses. In other words, longitudinal data could provide answers to questions of systematic individual differences between PWS infants in terms of change as a consequence of training combined with GH treatment.

Another aim of the RCT was to gain insight into the relationship between muscle strength, muscle mass, and motor performance in PWS infants. Therefore, there was a need to measure muscle strength in PWS infants objectively. Because objective measurement methods were lacking and little knowledge was available about muscle strength in infants in general, let alone in infants with motor problems, we developed a new measurement instrument to quantify muscle strength in infants and toddlers, the “Infant Muscle Strength meter” (IMS-meter).

Although muscle thickness was found to be strongly related to muscle strength and motor performance in PWS infants, some findings did not fit the explanation that motor problems in PWS infants are mainly caused by abnormal body composition. Among other findings, the fact that motor problems are at their worst in PWS infancy even though body composition is only mildly effected during that time.

Implications for the scientific field

This thesis revealed detailed insight into the motor development pattern of PWS in infancy and a significant clinically relevant effect of child-specific physical training combined with GH treatment on muscle thickness and motor development. Long-term longitudinal research is needed to determine how motor development further progresses and to evaluate the long-term effects of early intervention with GH treatment and training on general development. The underlying causes of motor problems in PWS should be studied more extensively. Findings of hypo-excitability of cortical motor areas in PWS adults, a possible relationship between brain abnormalities and hypotonia in PWS, and the PWS Necdin-deficient mouse model with a decreased number of motor neurons at birth all suggest innate central nervous system defects.

Implications for clinical practice

Based on our results, we recommended physical training combined with GH treatment in PWS infancy, taking into account muscle strength and skill learning in relation to growth and development. In pediatric physical therapy intervention it is a challenge to reach a balance between support in handling gravity and activating motor development to open up new opportunities to interact and learn from the environment. To increase muscle strength and improve motor skills, physical training should be an integrated part of daily life. Therefore, intervention in PWS infants should be focused on empowering the parents how to support their infant by providing a playful learning environment attuned to its constrained motor abilities.

Samenvatting

Dit proefschrift bevat een aantal studies die uitgevoerd zijn om inzicht te krijgen in de motorische ontwikkeling van zuigelingen en peuters met het Prader–Willi syndroom (PWS). De typische motorische kenmerken, de problemen die zij daarvan ondervinden en de invloed van interventies worden er in beschreven.

PWS is een zeldzame genetische afwijking, die wordt gekenmerkt door hypotonie, hyperfagie, overgewicht, een kleine gestalte, milde karakteristieke gezichtskenmerken, cognitieve beperkingen, gedragsproblemen en endocrinologische afwijkingen zoals hypogonadisme en groeihormoondeficiëntie. Op de zuigelingenleeftijd leidt de achterblijvende groei als gevolg van voedingsproblemen, ernstige hypotonie en spierzwakte tot een vertraagde psychomotorische ontwikkeling. Kinderen met PWS hebben gespecialiseerde, multidisciplinaire hulp nodig. Ook de ouders hebben behoefte aan gespecialiseerde begeleiding bij de opvoeding van hun kind. Omdat het syndroom weinig voorkomt, zijn de meeste zorgprofessionals er niet of onvoldoende mee bekend. In Nederland is gespecialiseerde kennis over PWS geconcentreerd in een aantal expertisecentra. Een van deze centra is gevestigd in het Radboud universitair medisch centrum in Nijmegen. In het Radboudumc ontstond behoefte aan meer wetenschappelijke onderbouwing van de zorg voor zuigelingen en peuters met PWS. Dit was de aanleiding voor de studies die uitgevoerd zijn in het kader van deze promotie.
Het doel was drieledig: 1) Kennisvergroting van de motorische ontwikkeling van jonge kinderen met PWS, 2) Inzicht krijgen in het effect van fysieke training gecombineerd met groeihormoon-behandeling op spiermassa, spierkracht en de motorische ontwikkeling en 3) Kwaliteitsverbetering van de zorg aan zuigelingen en peuters met PWS en hun ouders door het ontwikkelen van een interventieprogramma.

Vijf onderzoeksvragen werden geformuleerd:
1. Wat is er in de literatuur beschreven over de motorische problemen bij PWS, over de onderliggende oorzaken en over de effecten van interventies om de motoriek te verbeteren?
2. Wat is kenmerkend voor de motorische ontwikkeling van zuigelingen en peuters met PWS?
3. Wat is het effect van kind-specifieke, fysieke training gecombineerd met groeihormoon-behandeling op de motorische ontwikkeling van zuigelingen en peuters met PWS?
4. Kan spierkracht objectief vastgesteld worden bij normaal ontwikkelende zuigelingen en peuters en bij zuigelingen en peuters met PWS?
5. Wat is de relatie tussen groeihormoonbehandeling, spiermassa, spierkracht en motorische ontwikkeling bij zuigelingen en peuters met PWS?

Hoofdstuk 1

Ondanks dat motorische problemen bij zuigelingen met PWS kenmerkend zijn voor het syndroom en deze de ouders grote zorgen baren, bestaan er geen studies die zich specifiek richten op de motorische ontwikkeling van deze kinderen. Hierdoor is de kennis over het motorisch ontwikkelingspatroon van zuigelingen en peuters met PWS gering en is er beperkt inzicht in de effecten van interventies om de motorische ontwikkeling bij PWS te verbeteren. Dit proefschrift richt zich dan ook op de motorische ontwikkeling van zuigelingen en peuters met PWS en het effect van kind-specifieke fysieke training gecombineerd met GROEIHORMOON behandeling op de motorische ontwikkeling.

PWS wordt gekenmerkt door een variëteit aan fysieke, cognitieve en gedragsproblemen. Het syndroom wordt veroorzaakt door de afwezigheid van een stukje erfelijke informatie afkomstig van de vader op chromosoom 15q11-13. Bij PWS zijn verschillende neurologische afwijkingen in de structuur en het functioneren van de hypothalamus vastgesteld. Veel kenmerken van het syndroom worden in verband gebracht met deze hypothalame afwijkingen, waaronder de verstoorde hormoonregulatie, de hypoventilatie tijdens de slaap, slaap apneu, slaperigheid overdag, voedingsproblemen, verminderd verzadigingsgevoel, abnormale ademhalingsreacties en REM-slaap, en gestoorde temperatuurregulatie. Op de zuigelingenleeftijd zijn hypotonie, voedingsproblemen en een vertraagde psychomotorische ontwikkeling kenmerkend. Vanaf de kleuterleeftijd staan hyperfagie, gecombineerd met cognitieve, sociale en gedragsproblemen op de voorgrond. Tijdens de adolescentie is de puberale ontwikkeling onvolledig en op volwassen leeftijd komen cognitieve, sociale en psychiatrische problemen voor. Meer dan de helft van de patiënten met PWS ontwikkelt een scoliose.

Het kind-specifieke fysieke trainingsprogramma dat gebruikt is bij de studies is gebaseerd op een dynamisch systeembenadering. Vanuit dit perspectief is ontwikkelingsgerelateerde verandering in het motorisch repertoire het resultaat van een complexe interactie tussen het kind, de omgeving en doelgerichte acties of de taak. Studies naar de stapreflex (de stappende bewegingen die pasgeborenen maken wanneer ze rechtop gehouden worden) demonstreerden dat de taak (het stappen) beïnvloed werd door aan groei gerelateerde veranderingen in lichaamssamenstelling (de verhouding tussen vet- en spiermassa) en de omgeving, met name in relatie tot de zwaartekracht. Vanuit dit perspectief zijn hypotonie en spierzwakte bij zuigelingen met PWS beperkend bij het overwinnen van de zwaartekracht en het voldoende kunnen herhalen van bewegingen om een leereffect te kunnen realiseren. Interventies zouden gericht moeten zijn op het zodanig manipuleren van de taak en de omgeving dat het kind de mogelijkheid krijgt zijn biomechanische eigenschappen te exploreren en tegelijkertijd te leren om zijn beperkte motorische mogelijkheden op een functionele manier optimaal te gebruiken.

De motorische problemen die zuigelingen en peuters met PWS in het dagelijks leven ondervinden, de uitdagingen voor de ouders en verzorgers en elementen uit het interventieprogramma worden geïllustreerd in een klinische gevalsbeschrijving in Hoofdstuk 1, Boxen 1-4.

Hoofdstuk 2 en 3

In vier uitgebreide databestanden is systematisch naar literatuur gezocht (1956-2011) om een overzicht te krijgen van de actuele kennis over de motorische problemen bij PWS, over de onderliggende oorzaken daarvan en over interventies om de motoriek bij PWS te verbeteren. De resultaten zijn verwerkt in twee literatuuroverzichtsartikelen (systematic reviews). De eerste betreft de veronderstelde onderliggende oorzaken van de motorische problemen bij PWS en de tweede de motorische problemen en interventies om de motoriek te verbeteren.

Hoofdstuk 2

De motorische problemen bij PWS worden waarschijnlijk veroorzaakt door een afwijkende lichaamssamenstelling, waarbij het vetpercentage verhoogd is en de hoeveelheid spiermassa verminderd en mogelijk ook door neuromusculaire afwijkingen. De systematische literatuurstudie leverde 38 artikelen op: negen over lichaamssamenstelling, zeven over het neuromusculair systeem en 22 over de effecten van groeihormoonbehandeling op lichaamssamenstelling. In alle studies over lichaamssamenstelling werd vermeld dat bij mensen met PWS het vetpercentage verhoogd en de spiermassa verminderd is. Op de zuigelingenleeftijd is de lichaamssamenstelling al enigszins uit balans, maar de verhouding tussen vetmassa en spiermassa raakt steeds meer uit balans als de patiënten ouder worden. Zuigelingen hebben normaal ongeveer 24% vetmassa, in tegenstelling tot zuigelingen met PWS die 28 tot 32% vetmassa hebben. Het normale vetpercentage van kinderen is ongeveer 18%. Daarentegen ligt het bij kinderen met PWS tussen de 36 en 55%. De vetvrije massa (*lean body massa* (LBM)), die voornamelijk uit spiermassa bestaat, ligt normaal rond de 80%. Bij kinderen met PWS bedraagt dit echter 50 tot 60% van de totale lichaamsmassa. Op basis van deze bevindingen konden we vaststellen dat spiermassa ongeveer 25 tot 37% verminderd is bij mensen met PWS, wat de spierzwakte zou kunnen verklaren. Opmerkelijk is dat de motorische problemen het grootst zijn op de zuigelingenleeftijd, maar dat de lichaamssamenstelling op deze leeftijd slechts licht afwijkend is, waardoor het waarschijnlijk is dat ook neuromusculaire afwijkingen bijdragen aan de motorische problemen. Er waren maar enkele studies gericht op het neuromusculaire systeem en deze beschreven structurele en functionele spierafwijkingen bij PWS en hypoactiviteit van de motorische cortex. Het is onduidelijk of deze afwijkingen het resultaat zijn van aangeboren pathologie, verminderd spiergebruik of een combinatie van beide.

Alle groeihormoonstudies beschreven positieve effecten van groeihormoonbehandeling op lichaamssamenstelling. Onder invloed van groeihormoonbehandeling daalde het vetpercentage en nam LBM toe. Omdat groeihormoon ook leidt tot een versnelling van de lengtegroei, zou LBM gecorrigeerd moeten worden voor lichaamslengte. Studies waarin was gecorrigeerd, rapporteerden dat LBM afneemt bij kinderen met PWS, maar onder invloed van groeihormoon-behandeling stabiliseert. Daarentegen was er bij zuigelingen en peuters met PWS wel een toename van de hoeveelheid LBM gecorrigeerd voor lengte. Aangezien fysieke training de lichaamssamenstelling bij mensen met PWS verbetert en mogelijk een afname in spiermassa als

gevolg van verminderd spiergebruik zou kunnen voorkomen, hebben we verondersteld dat een combinatie van fysieke training en groeihormoon elkaar zouden kunnen versterken.

Hoofdstuk 3

Hoewel motorische problemen bij PWS kenmerkend zijn, is er weinig bekend over factoren die van belang zijn voor de behandeling. Deze studie werd uitgevoerd om een overzicht te geven van de kenmerken en prevalentie van de motorische problemen bij PWS en om de effecten van groeihormoonbehandeling en fysieke training op de motoriek te beschrijven. De systematische literatuurstudie leverde 34 artikelen op: 13 over motoriek, 12 over de effecten van groeihormoonbehandeling op de motoriek en negen over de effecten van fysieke training.

Bij zuigelingen en peuters is het motorisch ontwikkelingspercentage[1] 30–57%. Bij kinderen en volwassenen met PWS is het fysieke activiteitenniveau, de fysieke fitheid en de spierkracht verminderd. Bij volwassenen met PWS is de balanscapaciteit verminderd en is het looppatroon abnormaal. Op basis van deze bevindingen concludeerden we dat de motorische ontwikkeling van zuigelingen en peuters met PWS longitudinaal onderzocht zou moeten worden en dat motorische problemen bij kinderen en volwassenen met PWS bestudeerd zouden moeten worden in relatie tot structurele en functionele beperkingen gerelateerd aan de consequenties voor het dagelijks leven.

De meeste studies beschreven positieve effecten van groeihormoonbehandeling op de motorische ontwikkeling bij zuigelingen en peuters met PWS. Ook bij kinderen en volwassenen met PWS werden positieve effecten van groeihormoonbehandeling op de motoriek gevonden. Alle trainingsstudies bij kinderen en volwassenen met PWS meldden positieve effecten van fysieke training op gewicht, lichaamssamenstelling, het fysieke activiteitenniveau en/of fysieke fitheid. We concludeerden dat fysieke activiteit en spierkrachttraining een belangrijk onderdeel behoren te zijn in het dagelijks leven van mensen met PWS en adviseerden een combinatie van groeihormoonbehandeling en fysieke training zo vroeg mogelijk in de ontwikkeling.

Hoofdstuk 4

Ernstige hypotonie, spierzwakte en een vertraagde motorische ontwikkeling zijn kenmerkend voor zuigelingen en peuters met PWS. Interventies om de motorische ontwikkeling te verbeteren zijn met name gericht op groeihormoonbehandeling. Hoewel fysieke training bij kinderen en volwassenen met PWS tot positieve resultaten leidde, zijn er geen onderzoeken bekend over de effecten van fysieke training bij zuigelingen en peuters met PWS. Het motorische ontwikkelingspatroon van zuigelingen en peuters met PWS is nog niet onderzocht en de effecten van groeihormoonbehandeling op de motorische ontwikkeling zijn nog niet eerder onderzocht in relatie tot het ontwikkelingspatroon. Daarom hebben wij in een longitudinale studie bekeken of groeihormoonbehandeling het effect van fysieke training op de motorische ontwikkeling bij

[1] Motorisch ontwikkelingspercentage = motorische ontwikkelingsleeftijd/chronologische leeftijd × 100.

zuigelingen en peuters met PWS versterkte. In ditzelfde cohort hebben we ook een studie gedaan naar de effecten van fysieke training gecombineerd met groeihormoonbehandeling op spierdikte en de relatie tussen spierdikte, spierkracht en motorische ontwikkeling. De resultaten van deze spierstudie zijn verwerkt in hoofdstuk 6.

Tweeëntwintig zuigelingen en peuters met PWS werden twee jaar gevolgd in een gerandomiseerde en gecontroleerde studie (randomized controlled trial, RCT). Beide groepen volgden een kind-specifiek fysiek trainingsprogramma vanaf de start van de het onderzoek. De behandelgroep (n=10) startte groeihormoonbehandeling nadat de baselinemetingen uitgevoerd waren en de controle groep (n=12) startte groeihormoonbehandeling na een controleperiode. Elke drie maanden werd het motorisch ontwikkelingsniveau gemeten met behulp van drie gestandaardiseerde motorische testen: de Alberta Infant Motor Scale (AIMS), de Motor Scale of the Bayley Scales of Infant and Toddler Development 2nd edition (BSID-II), en de Gross Motor Function Measure (GMFM).

We ontdekten dat zuigelingen en peuters met PWS gemiddeld twee keer zo lang doen over het bereiken van de motorische mijlpalen in vergelijking tot kinderen met een normale ontwikkeling. We vonden ook dat de snelheid van de motorische ontwikkeling afnam in de tijd en leek te stagneren. De analyses (multilevel regression analysis) lieten zien dat de motorische ontwikkelingspatronen significant tussen de kinderen verschilden. Dit kon gedeeltelijk verklaard worden door het motorische ontwikkelingsniveau bij baseline en het effect van groeihormoon-behandeling. Kinderen met een grotere motorische achterstand bij de start van het onderzoek ontwikkelden zich langzamer dan kinderen die een kleinere achterstand hadden aan het begin van het onderzoek. Fysieke training gecombineerd met groeihormoonbehandeling had een positief en klinisch relevant effect op de motorische ontwikkeling. Het statistisch model van de AIMS (AIMS multilevel model) voorspelde dat kinderen die fysieke training kregen en behandeld zijn met groeihormoon gemiddeld genomen het einde van de test 3,8 maanden eerder bereikten dan kinderen die fysieke training kregen zonder groeihormoonbehandeling. Daarnaast voorspelde het GMFM multilevel model dat peuters met PWS die fysieke training gecombineerd met groeihormoonbehandeling kregen, aan het eind van de studie kunnen rennen, over een stok kunnen stappen op kniehoogte en vanuit rugligging kunnen opkomen naar een zittende positie zonder het hoofd te laten hangen. Zonder groeihormoonbehandeling zijn de peuters wel in staat om te lopen en een paar seconden op een been te staan, maar ze kunnen nog niet rennen en laten het hoofd hangen bij het opkomen vanuit rugligging. Ook vonden we aanwijzingen dat het op jonge leeftijd starten van groeihormoonbehandeling leidt tot een eerdere start van de versnelling in de motorische ontwikkeling en over de tijd tot een hoger motorisch niveau.

Gebaseerd op deze resultaten concludeerden we dat groeihormoonbehandeling het effect van fysieke training op de motorische ontwikkeling van zuigelingen en peuters met PWS versterkte, maar dat er ondanks dit positieve effect een grote motorische achterstand blijft bestaan. Daarnaast leek het gunstige effect groter wanneer groeihormoon op een jonge leeftijd gestart werd.

Hoofdstuk 5

Het was bij aanvang van de studie niet mogelijk om bij zuigelingen en peuters spierkracht objectief vast te stellen, omdat er geen geschikte meetinstrumenten waren voor zeer jonge kinderen. De diagnostisering van een zuigeling met een vertraagde motorische ontwikkeling, spierzwakte en hypotonie zou verbeteren als spierkracht geobjectiveerd kon worden, aangezien hypotonie gecombineerd met ernstige spierzwakte wijst op een perifere oorzaak en hypotonie met relatief milde spierzwakte op een centrale oorzaak. Als spierkracht gekwantificeerd kon worden, zou het ook mogelijk zijn om interventies om spierkracht te verbeteren te evalueren. Daarom hebben we een methode ontwikkeld om spierkracht bij jonge kinderen objectief te meten, met behulp van de *infant muscle strength meter* (IMS-meter). We gebruikten daarbij een zogenaamde 'trektaak'. De trektaak was gebaseerd op trekactiviteiten die kinderen vanaf zes maanden oud spontaan kunnen laten zien, wanneer ze een object naar zich toe proberen te halen. Het doel van dit onderzoek was om vast te kunnen stellen of het mogelijk was om maximale spierkracht betrouwbaar te meten bij normaal ontwikkelende kinderen en kinderen met PWS, wanneer gebruik werd gemaakt van spontane trekacties. Daarmee wilden we referentiedata verzamelen om een model te ontwikkelen waarmee spierkracht voorspeld kon worden.

Eenentachtig normaal ontwikkelende kinderen van 6-36 maanden oud en 17 kinderen met PWS van 24 maanden oud werden geïncludeerd. In een subgroep van 46 normaal ontwikkelende kinderen werd spierkracht twee keer gemeten door twee verschillende onderzoekers. Hiermee kon worden vastgesteld dat de *interrater* betrouwbaarheid van de IMS-meter hoog was. De convergent validiteit van de IMS-meter werd vastgesteld door de sterke correlatie tussen spierkracht, leeftijd, lengte en gewicht. Een multiple lineair regressiemodel werd ontwikkeld om spierkracht op basis van leeftijd, lengte en gewicht te kunnen voorspellen, welke 73% van de variatie tussen normaal ontwikkelende kinderen verklaarde. Bij kinderen met PWS was de spierkracht significant lager, maar de variatie tussen kinderen was ook hoger en drie kinderen met PWS hadden een normale spierkracht. Bij kinderen met PWS bleek spierkracht een beperkende factor voor de motorische ontwikkeling, aangezien kinderen, waarbij spierkracht meer was verminderd, een grotere motorische achterstand hadden dan kinderen waarbij spierkracht minder was aangedaan. Meer referentiedata zouden verzameld moeten worden om het voorspellen van spierkracht te optimaliseren. Daarnaast zou de IMS-meter in andere patiëntengroepen getest moeten worden en zijn technische verbeteringen nodig om het meetinstrument geschikt te maken voor de klinische praktijk.

Hoofdstuk 6

Bij PWS heeft groeihormoonbehandeling een positief effect op de lichaamssamenstelling en de motoriek. Hoewel er wordt verondersteld dat groeihormoonbehandeling bij zuigelingen en peuters met PWS de spierdikte verbetert en daarmee spierkracht verhoogt en de motorische ontwikkeling verbetert, ontbreken studies die deze samenhang demonstreren. Fysieke training

heeft ook een positief effect op lichaamssamenstelling en motoriek bij PWS. Daarom werd in deze longitudinale studie bij zuigelingen en peuters met PWS het effect van kind-specifieke fysieke training gecombineerd met groeihormoonbehandeling, op spierdikte onderzocht en gekeken of er een relatie was tussen spierkracht en motorische ontwikkeling.
Tweeëntwintig zuigelingen en peuters met PWS werden twee jaar gevolgd in een gerandomiseerde en gecontroleerde studie (randomized controlled trial, RCT) met een behandelgroep (n=10) en wachtlijst controle groep (n=12). Spierdikte van vier spiergroepen (linkerbiceps brachii, rechterbovenarm flexoren, rechterquadriceps, linkertibialis anterior) werden gemeten met behulp van spierechografieën. Spierkracht werd gemeten met de IMS-meter en het motorische ontwikkelingsniveau werd vastgesteld met de *Gross Motor Function Measure* (GMFM). Spierdikte was in alle onderzochte spiergroepen verminderd, waarbij de structuur van de spieren normaal was op basis van de echo-intensiteit metingen. Spierdikte verbeterde onder invloed van fysieke training gecombineerd met groeihormoonbehandeling, tot de lage normale range. Spierdiktetoename was sneller in spieren die frequenter gebruikt werden tijdens de vroege motorische ontwikkeling. Aangezien dit effect onafhankelijk was van groeihormoon wijst dit op een positief effect van fysieke training. Bovendien werd er voor het eerst aangetoond dat bij zuigelingen en peuters met PWS spierdikte sterk gerelateerd is aan verminderde spierkracht en een motorische ontwikkelingsachterstand. Echter, hoewel spierdikte toenam tot een lage normale range, bleef de motorische ontwikkeling sterk achter ten opzichte van de normale ontwikkeling.

Hoofdstuk 7

We gebruikten de *International Classification of Functioning, Disability and Health; Children and Youth version* (ICF-CY) of de *World Health Organization* [2008], om de in de literatuur gerapporteerde motorische problemen bij PWS en de onderliggende oorzaken van de problemen te categoriseren. Hieruit bleek dat de focus voornamelijk lag op het niveau van lichaamsfuncties (*body functions*) en lichaamsstructuren (body structures) en dat sommige bevindingen minder aandacht hebben gekregen.
Het is evident dat zuigelingen en peuters met PWS door ernstige hypotonie, verminderde spierkracht en afwijkende lichaamssamenstelling niet optimaal zijn toegerust om te bewegen. Daarom benaderden we de motorische problemen op het ICF-CY niveau van activiteiten en participatie. In de normale motorische ontwikkeling is lichaamssamenstelling een bepalende factor bij het kunnen overwinnen van de zwaartekracht. Verschillende experimenten, met een dynamisch systeemperspectief op de motorische ontwikkeling, lieten zien dat door het manipuleren van de oriëntatie van het lichaam op een manier dat de zwaartekracht minder invloed heeft op het lichaam, zuigelingen beter in staat zijn bepaalde motorische taken uit te voeren. Op basis hiervan suggereerden we dat het mogelijk kon zijn om de motorische leercondities voor zuigelingen en peuters met PWS te verbeteren, waardoor ze beter in staat zouden zijn om vaardigheden te oefenen en nieuwe vaardigheden te leren.

We suggereerden dat het effect van groeihormoonbehandeling op de motorische ontwikkeling verbeterd zou kunnen worden als de zuigelingen en peuters tegelijkertijd gestimuleerd zouden worden om motorische vaardigheden daadwerkelijk te oefenen. Daarom werd een kind-specifiek fysiek trainingsprogramma ontwikkeld waarin de kinderfysiotherapeutische begeleiding transmuraal georganiseerd was. De ouders en de kinderfysiotherapeuten van de families werden gecoached door kinderfysiotherapeuten van het PWS expertisecentrum (Radboudumc). Om de effectiviteit van training gecombineerd met groeihormoonbehandeling te kunnen onderzoeken, voerden we een longitudinale gerandomiseerde en gecontroleerde studie (randomized controlled trial, RCT) uit. Vanaf het begin van de studie participeerden alle kinderen in het trainingsprogramma. De behandelgroep startte groeihormoon direct na de baseline metingen en de controlegroep startte groeihormoonbehandeling na een controleperiode van zes maanden. Door het longitudinale onderzoek kregen we informatie over de ontwikkelingspatronen van de kinderen; hoe de motorische testuitslagen veranderden over de tijd binnen elk individueel kind (*within-individual change*). Gebaseerd op deze informatie is het mogelijk om te bekijken of verschillende kinderen andere patronen van ontwikkeling laten zien en om factoren te detecteren die deze verschillen kunnen voorspellen, door gebruik te maken van *multilevel analyses*. Longitudinale data kunnen antwoorden geven op vragen over systematische, individuele verschillen tussen zuigelingen en peuters met PWS in termen van verandering als een consequentie van fysieke training gecombineerd met groeihormoonbehandeling.

De longitudinale studie had ook als doel om inzicht te krijgen in de relatie tussen spiermassa, spierkracht en motorische ontwikkeling bij zuigelingen en peuters met PWS. Om deze reden was het noodzakelijk om spierkracht objectief te meten bij zuigelingen en peuters met PWS. Omdat objectieve meetmethodes om spierkracht bij jonge kinderen te meten ontbraken en er in zijn algemeenheid weinig bekend was over spierkracht bij zuigelingen en peuters, in het bijzonder met motorische problemen, hebben we een nieuw meetinstrument ontwikkeld: de *Infant Muscle Strength meter* (IMS-meter).

Hoewel bleek dat spierdikte sterk was gerelateerd aan spierkracht en motorische ontwikkeling bij zuigelingen en peuters met PWS, waren er enkele bevindingen die niet in overeenstemming zijn met de verklaring dat motorische problemen bij PWS voornamelijk worden veroorzaakt door een afwijkende lichaamssamenstelling, waaronder het feit dat motorische problemen het ernstigste zijn op de zuigelingenleeftijd, terwijl op die leeftijd lichaamssamenstelling enkel mild afwijkend is. Het is mogelijk dat ook neuromusculaire afwijkingen bijdragen aan de motorische problemen. De bevindingen van hypoactiviteit van de motorische cortex bij volwassenen met PWS, een mogelijke relatie tussen corticale afwijkingen en hypotonie bij PWS en het PWS Necdine-deficiënt muismodel met een verminderde hoeveelheid motorneuronen suggereren aangeboren afwijkingen in het centrale zenuwstelsel.

Implicaties voor de wetenschap

Dit proefschrift geeft gedetailleerde informatie over het motorisch ontwikkelingspatroon van zuigelingen en peuters met PWS en toonde een significant en klinisch relevant effect aan van kind-specifieke fysieke training gecombineerd met groeihormoonbehandeling op spierdikte en motorische ontwikkeling. Langlopend longitudinaal onderzoek is nodig om te kunnen vaststellen hoe de motorische ontwikkeling verder verloopt en om de langetermijneffecten van vroege interventie met groeihormoon en fysieke training te kunnen evalueren. De onderliggende oorzaken van de motorische problemen bij PWS zouden uitgebreider onderzocht moeten worden.

Implicaties voor de klinische praktijk

Gebaseerd op deze bevindingen adviseren we fysieke training gecombineerd met groeihormoon-behandeling te starten op de zuigelingenleeftijd. Bij de fysieke training moeten zowel spierkracht training als vaardigheidstraining in relatie tot ontwikkeling in acht genomen worden. Bij kinderfysiotherapeutische interventies is het een uitdaging om een balans te vinden tussen het ondersteunen van het lichaam met het oog op de zwaartekracht en het stimuleren van de motorische ontwikkeling om nieuwe mogelijkheden te openen om interactie aan te gaan met de omgeving en te leren van de omgeving. Om spierkracht en motorische vaardigheden te verbeteren zou fysieke training een geïntegreerd onderdeel moeten zijn van het dagelijks leven. Daarom moeten interventies bij zuigelingen en peuters met PWS ook gericht zijn op de ouders. Zij moeten leren hoe ze hun kind kunnen stimuleren door het creëren van een leeromgeving welke afgestemd is op de motorische mogelijkheden van hun kind.

Dankwoord

Wat begon als een klein onderzoeksproject groeide uit tot een groter project en leidde uiteindelijk tot dit proefschrift en mijn promotie. Tijdens het onderzoeksproject kreeg ik de kans om de motorische ontwikkeling van 22 jonge kinderen met het Prader Willi syndroom (PWS) twee jaar te volgen. Elke drie maanden was ik getuige van de stappen die deze kinderen maakten in hun ontwikkeling en was ik samen met ouders en kinderfysiotherapeuten enthousiast over de motorische mijlpalen die bereikt werden. Ik mocht me verdiepen in de motorische problemen die spelen bij PWS en de achterliggende oorzaken. Voor mij was het een prachtig project waarin een fijne afwisseling bestond tussen gedegen onderzoekswerk en het helpen ontwikkelen en uitvoeren van een interventieprogramma dat als doel had de motorische ontwikkeling van kinderen met PWS zo optimaal mogelijk te begeleiden. Ik heb met veel toewijding, interesse en plezier gewerkt, maar ik had het onderzoek natuurlijk nooit zonder de hulp van anderen kunnen uitvoeren. De belangrijkste betrokkenen bij het project wil ik graag op deze plaats danken.

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Veel verschillende coauteurs hebben mij met hun expertise bijgestaan.
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Linda

Curriculum vitae

Linda Reus werd geboren op 12 december 1976 in Amsterdam. Als jong meisje droomde ze van een carrière als balletdanseres. Het was dan ook niet verwonderlijk dat ze na haar HAVO-diploma in 1995 aan de Open School Bijlmer (OSB) in Amsterdam een opleiding volgde tot docent dans aan de Fontys dansacademie in Tilburg. Door haar ervaringen met dans in het amateurveld bij het lesgeven aan peuters, kleuters, kinderen en volwassenen en haar ervaringen als dansdocent binnen het voorgezet onderwijs raakte Linda wetenschappelijk geïnteresseerd in motoriek, leren en ontwikkeling. Daarom startte ze in 2000 met haar studie Psychologie aan de Radboud Universiteit in Nijmegen. In 2006 behaalde zij haar master Cognitiewetenschap met een vrije afstudeervariant in motoriek en ontwikkeling. Haar afstudeeronderzoek deed ze aan de afdeling Kinderfysiotherapie van het Radboud universitair medisch centrum. Haar onderzoek ging over het vermogen van kleuters om in te kunnen schatten tot hoever ze kunnen reiken en of dit wordt beïnvloed door een transitie in lichaamshouding die veel kleuters doormaken. Tijdens haar studie raakte Linda bekend met de Dynamische Systeem theorie, een benadering van ontwikkeling die haar enorm inspireerde. In 2006 startte het onderzoek naar de motorische ontwikkeling van zuigelingen en peuters met het Prader-Willi syndroom aan de afdeling Kinderfysiotherapie van het Radboud universitair medisch centrum. In 2008 kon het onderzoek, dankzij een aanvullende subsidie van het Prader Willi Fonds, uitgebouwd worden naar een promotietraject. Tijdens het onderzoek volgde Linda de motorische ontwikkeling van 22 zuigelingen en peuters met het Prader-Willi syndroom gedurende twee jaar. Elke drie maanden was zij getuige van de stappen die de kinderen maakten in hun ontwikkeling. Mede door deze ervaringen raakte zij ook geïnteresseerd in de sociaal emotionele ontwikkeling. Daarom startte Linda in 2013 een cursus klinische vaardigheden aan de opleiding Pedagogische Wetenschappen van de Radboud Universiteit Nijmegen, waarin ze leerde werken met de behandelmethodiek *Emerging Body Language*. Vanaf 2014 is Linda ook werkzaam als docent aan de opleiding Pedagogische Wetenschappen van de Radboud Universiteit Nijmegen.

Linda is getrouwd met Adriaan van Liempt. Samen hebben ze twee kinderen Mannik (2010) en Noek (2013).