Postprandial cardiac autonomic function in Prader–Willi syndrome

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Summary

Context
Individuals with Prader–Willi syndrome (PWS) have a high cardiovascular risk, the mechanism of which is unclear. There may be dysfunction in the autonomic nervous system (ANS) in PWS.
Objective
To measure, as indicators of cardiac autonomic function, postprandial heart rate variability (HRV) and arterial stiffness in adults with PWS.
Methods
Ten adults with PWS were compared with 11 matched healthy obese subjects and 9 healthy lean subjects. Electrocardiographic traces and arterial stiffness were recorded over a period of 10 minutes at −60, 0, 30, 60, 120 and 240 minutes after consumption of a standardized 600-kCal breakfast. Frequency domain analysis was performed using fast Fourier transform to estimate power spectral density in the full spectrum and in low-frequency (LF 0·04–0·15 Hz) and high-frequency (HF 0·15–0·40 Hz) bands.
Results
ANCOVA revealed a reduced LF HRV meal response in adults with PWS compared with obese controls, with no differences in HF HRV, LF/HF ratio, heart rate, total power or arterial stiffness meal responses.
Conclusions
This study assessed cardiac autonomic function in adults with PWS compared with matched obese and lean subjects in response to a meal. Results suggest impaired postprandial ANS responsiveness in PWS, which could contribute to both the known increased cardiovascular risk and obesity.

Introduction

Prader–Willi syndrome (PWS) is the most common genetic form of obesity with a prevalence of 1:25 000–1:27 000 live births.[**1, 2**] It is presumed that this imprinting disorder of chromosome 15 results in dysfunction of several hypothalamic centres. Characteristic syndromic features include hyperphagia, intellectual disability, behavioural abnormalities and endocrine disorders such as growth hormone (GH) deficiency, hypogonadism and hypoinsulinaemia.

Obesity appears to be the main cause of increased morbidity and early mortality in individuals with PWS, who are at high risk of cardiovascular disease[**3**] despite reported lower visceral fat tissue[**4**] and increased insulin sensitivity compared with healthy obese subjects.[**5**]

Computer-assisted analysis of heart rate variability (HRV), the variation in the interval between pulses, can detect alterations in the autonomic nervous system (ANS) which have been associated with obesity. However, it is still unclear whether obesity itself causes these impairments or whether an autonomic disorder is partly responsible for obesity.[**6**]

Diminished activity of the parasympathetic nervous system has been postulated in PWS by DiMario *et al*. based on the measurement of pupillary reactions,[**7, 8**] while results of measures of autonomic cardiac activity have been inconsistent.[**9, 10**] Further investigation is warranted, because impaired vagal activity is associated not only with increased mortality[**11**]

but also with the absence of hormone- or gastric distension–induced satiety in rats.[**12-14**] If such a disturbance is demonstrable, it could suggest a novel target for future therapies for PWS such as vagal nerve stimulation.[**15**]

Vascular function, assessed by arterial stiffness, is an independent predictor of total and cardiovascular mortality.[**16**] Patel *et al*. described microcirculatory dysfunction in PWS individuals but, compared with lean controls, reported no difference in fasting large arterial stiffness.[**17**] We have previously found fasting arterial stiffness in PWS and obese individuals to be similarly elevated compared with lean controls.[**18**] Given that increased AIx has been associated with GH deficiency,[**19**] a typical feature of PWS, this study will investigate whether postprandial arterial stiffness differs between PWS and obese individuals.

In most studies, PWS subjects have been matched with controls using body mass index (BMI). This may not be sufficiently accurate, as individuals with PWS are known to have a higher percentage of body fat and lower lean mass for a given BMI. To control for the contribution of obesity itself to the features found in PWS, we intended to study PWS subjects matched not only for BMI, but also for percentage of total body and central abdominal fat.

The aim of this study was to assess mechanisms of increased vascular risk in PWS by measuring HRV and arterial stiffness in response to meal-stimulated hyperinsulinaemia in comparison with both healthy obese and lean control subjects. By measuring HRV, differences in ANS activity were investigated, which could contribute to hyperphagia and other typical features of PWS. Furthermore, the evaluation of postprandial HRV response provides additional information about the relationship between the ANS and obesity.

Methods

Subjects

Adult patients with genetically confirmed PWS were recruited from the Prader–Willi Syndrome Clinic at the Royal Prince Alfred Hospital, Sydney, Australia. Obese and lean control subjects were recruited by advertising in the local newspaper, hospital and research institute. The study was approved by the Hospital Research and Ethics committee, and informed consent was obtained from all participants and/or their parents/guardians. Three of the PWS participants had type 2 diabetes (T2D) (treated with metformin alone, metformin and gliclazide, and metformin and Mixtard 30/70, respectively). Two obese controls had T2D (treated with metformin and gliclazide, and metformin, sitagliptin and rosiglitazone, respectively). On the day of the study, medications were withheld until study completion.

Study design

Subjects were required to fast from midnight before arriving at 8:30 a.m. at the Clinical Research Facility, Garvan Institute of Medical Research. The study meal consisted of a standardized 600-kcal breakfast (50% carbohydrates, 35% fat and 15% protein) of muesli, apple, banana, low-fat milk and natural yoghurt, which was eaten within 20 min.

Anthropometry

Weight was measured in a hospital gown and height assessed by stadiometer. Body mass index was calculated as body weight in kilograms divided by height in metres squared (kg/m2).

Dual-energy X-ray absorptiometry

Body composition was measured by dual-energy X-ray absorptiometry according to a three-compartmental model comprising fat mass, lean tissue and bone mineral content (Lunar DPX; GE-Lunar instrument, Madison, WI, USA). Total body fat was expressed as percentage of total body mass and central abdominal fat as percentage of total abdominal soft tissue.

Biochemical measures

Whole-blood glucose was determined by the glucose oxidase method using an YSI glucose analyser (model 2300 STAT PLUS 230V; YSI, Inc., Yellow Springs, OH, USA). Serum insulin was measured using a commercial radioimmunoassay (Linco, St. Charles, MO, USA).

Arterial stiffness and heart rate variability

Arterial stiffness and HRV were measured by SphygmoCor® (AtCor Medical, Sydney, NSW, Australia) pulse wave analysis and heart rate variability system. Two baseline measurements of

arterial stiffness and HRV were performed and averaged for analysis. Repeat measurements were taken at 30, 60, 120, 180 and 240 min after the meal.

Central arterial pressures were derived from noninvasive measurement of radial pulse waveforms using a highly sensitive transducer. Augmentation index (AIx) was used as measure of arterial stiffness and calculated as follows: augmented systolic pressure (due to the reflected wave by arterial walls) divided by the total pulse height ×100%. As reported previously by our group,[**6**] the day-to-day coefficient of variation for repeated fasting measurements of AIx on four separate days is 5·3% in our hands. Values were adjusted for age, sex and heart rate (75 bpm).

Heart rate variability was analysed according to the Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology.[**20**] ECG was recorded over a period of 10 min at each time point, and only stable periods without ectopic beats or missing data were used for analysis. Frequency domain analysis was performed using fast Fourier transform with the Hamming window to estimate power spectral density. Total power was divided into low frequency (LF 0·04–0·15 Hz) and high frequency (HF 0·15–0·40 Hz), expressed in absolute values (ms2).

Upon spectral analysis, HRV can be reduced to two main components: low frequency (LF; 0·04–0·15 Hz) and high frequency (HF; 0·15–0·40 Hz). While the HF component represents parasympathetic activity, the LF component is a measure of both sympathetic and parasympathetic activity. Thus, the ratio of LF to HF can be interpreted as an index of sympathovagal balance.[**20**]

Statistical analysis

All analyses were performed using jmp (version 4.0.1; SAS Institute Inc., Cary, NC, USA). All HRV data (total power, LF, HF and LF/HF) were analysed after natural logarithm transformation. Effects of group on baseline (premeal, average of −60 and 0 min data) measures were assessed by one-way anova. In preliminary analyses, effects of group on the HRV and AIx time course data (30–240 min) were analysed by manova with a baseline covariate. No effects of time during the meal were detected, and the time course data were therefore averaged over the period 30-240 min to provide meal response data for the main analyses. Effects of group on HR, HRV and AIx meal response data were analysed using ancova with baseline covariate. Pairwise between-group comparisons were restricted to planned contrasts between PWS and obese groups. Glucose and insulin meal responses were calculated as average postprandial values obtained from areas under the curves (AUC, 0–240 min) using the trapezoidal rule, divided by time. Glucose and insulin data were ln-transformed for analysis. Differences in subject characteristics between the PWS and obese groups were assessed using Dunnett's test. Two-way repeated-measures anova was used to assess differences in baseline and meal responses between groups, with repeats in time (baseline, average postprandial) and planned contrasts between PWS and obese groups; residuals from all models were normally distributed ($P > 0{\cdot}05$, Shapiro–Wilk W test). Data are expressed as mean ± standard error unless otherwise indicated. P-values <0·05 were considered statistically significant.

Fasting insulin and glucose were not different between groups (Fig. 2). There was no difference between PWS and Obese in postprandial insulin response (Fig. 2a), although the PWS group had a higher postprandial glucose response than Obese ($P = 0.04$; Fig. 2b).

Results

Baseline subject characteristics are summarized in Table 1. Ten adults with Prader–Willi syndrome, 11 obese control subjects and 9 lean controls were recruited. Groups were matched for age, sex and ethnicity, and PWS and obese groups were further matched for BMI. Percentage total and central fat, systolic blood pressure and diastolic blood pressure were not different between these two groups. Fasting glucose and HOMA-IR were unaffected by inclusion/exclusion of subjects with T2D.

Heart rate, HRV and arterial stiffness are shown in Table 2 and Fig. 1. In PWS, obese and Lean groups, HR increased after ingestion of the meal, with a peak at 60 minutes (data not shown). There were no differences in HR meal response between groups (Fig. 1a).

Table 1. Anthropometric characteristics

	Lean	Obese	Prader–Willi syndrome (PWS)
Age	28·9 ± 1·3	32·9 ± 2·5	27·9 ± 2·7
M/F	5/4	6/5	6/4
Height (cm)	168 ± 3	168 ± 2	155 ± 4†
Weight (kg)	60 ± 2	96 ± 2	88 ± 7
BMI (kg/m^2)	21·3 ± 0·5	34·3 ± 1·3	36·9 ± 2·9
% Total Fat	26·9 ± 2·9	44·2 ± 3·0	48·6 ± 2·8
% Abdominal Fat	26·2 ± 1·9	46·4 ± 2·4	46·3 ± 2·4
Systolic BP (mmHg)	121 ± 7	125 ± 3	130 ± 7
Diastolic BP (mmHg)	63 ± 2	69 ± 2	73 ± 2
Fasting glucose (mM)	4·4 ± 0·1	4·9 ± 0·4	4·8 ± 0·2
Fasting insulin (μU/l)	8·6 ± 0·6	15·1 ± 1·5	15·5 ± 2·3
HOMA-IR	1·7 ± 0·1	3·5 ± 0·7	3·4 ± 0·6

†$P \leq 0{\cdot}05$ PWS *vs* obese (ANOVA).

There were no differences between groups in total HRV meal response (Fig. 1b). Similarly, no differences were seen in the HF spectral band (Fig. 1d) or in LF/HF ratio (Fig. 1e). PWS group had a reduced LF response compared with obese group ($P = 0{\cdot}01$; Fig. 1c). Arterial stiffness was strongly affected by group at baseline ($P = 0{\cdot}006$; Table 2) but was not different between PWS and obese groups. There were no differences between groups in AIx meal response (Table 1F).

Discussion

This study demonstrated that while most parameters of cardiovascular and autonomic function measured did not differ between PWS subjects and obese controls, a reduced LF meal response was detected.

As the HF spectral band of HRV represents parasympathetic activity and there was no difference in postprandial HF between PWS subjects and obese controls, our study suggests that individuals with PWS have an adequate parasympathetic response to a meal stimulus. LF HRV is not a direct metric of sympathetic activity; instead, it represents both parasympathetic and sympathetic autonomic activities. However, as the HF HRV band showed parasympathetic activity to be not different between groups, it is possible that the detected reduction in the LF component in the PWS group may be largely due to an impairment in sympathetic meal response.

Previously, Wade *et al.*[**10**] found no evidence of altered cardiac function in PWS during orthostatic manoeuvres. In a 1994 study, DiMario *et al.*[**8**] detected a diminution in parasympathetic activity, only based, however, on analysis of pupillary contraction. The current study is the first to assess cardiac autonomic function in response to ingestion of a meal, which may explain why this study differs from previous investigations.

Heart rate variability is a robust indicator of cardiovascular risk – it strongly predicts mortality in postmyocardial infarction patients.[**21**] Considering that there is a well-documented propensity towards cardiovascular morbidity in adults with PWS,[**22-25**] it is of note that we detected no corresponding changes in total HRV, HF or LF/HF ratio. However, we postulate that the reduced LF responsiveness we saw in the PWS group may be related to increased cardiovascular risk.

We also found that another important cardiovascular risk factor, arterial stiffness, remained elevated in PWS and obese individuals compared with lean controls. Our data suggest that such changes in arterial stiffness accompany adiposity and are not a primary characteristic of PWS itself. Further investigation will show whether arterial stiffness is improved in PWS subjects receiving GH treatment.

Table 2. Baseline and meal cardiac autonomic parameters

	Units	Period	Lean	Obese	Prader–Willi syndrome (PWS)
HR	bpm	Baseline	63·0 ± 2·6	66·4 ± 3·7	64·7 ± 3·0
		Meal*	69·9 ± 3·4	71·5 ± 3·1	72·1 ± 2·8
Heart rate variability	ms^2†	Baseline	1710 (1426, 7133)	1726 (548, 4536)	3095 (432, 12477)
		Meal*	1648 (865, 3161)	1122 (342, 3505)	1076 (305, 5217)
Low-frequency (LF)	ms^2†	Baseline	1248 (558, 2949)	1011 (243, 2171)	656 (201, 2229)
		Meal	1658 (964, 2388)	583 (495, 2014)	470‡ (188, 1083)
High-frequency (HF)	ms^2†	Baseline	587 (412, 2478)	441 (88, 1725)	534 (108, 2004)
		Meal	492 (257, 1365)	496 (94, 876)	285 (88, 908)
LF/HF		Baseline	0·49 ± 0·23	0·86 ± 0·25	0·51 ± 0·18
		Meal	1·11 ± 0·21	1·27 ± 0·26	0·73 ± 0·20
AIx		Baseline§	2·3 ± 2·2	16·0 ± 2·5	17·1 ± 3·3
		Meal*	−2·4 ± 3·5	11·3 ± 3	16·0 ± 2·9

*Meal effect ($P \leq 0{\cdot}01$, RM MANOVA).
†Data presented as median (interquartile range).
‡PWS *vs* obese ($P \leq 0{\cdot}01$, ANCOVA).
§Effect of group at Baseline ($P \leq 0{\cdot}05$, ANOVA).

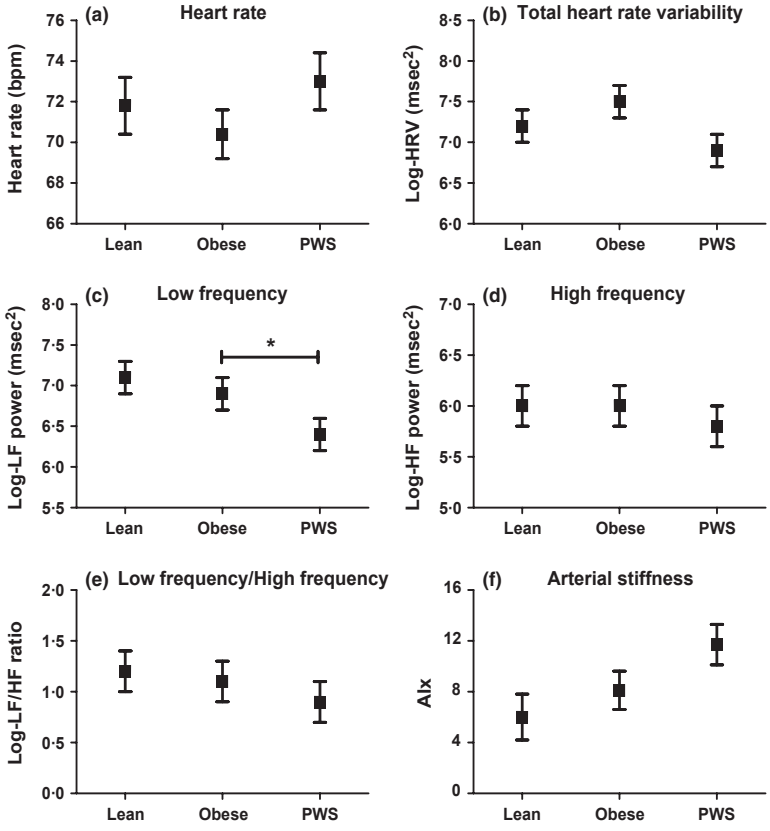

Fig. 1 Postprandial responses of heart rate (**a**), total heart rate variability (HRV; **b**), low-frequency (LF) HRV (**c**), high-frequency HRV (**d**), LF/high-frequency ratio (**e**) and arterial stiffness (**f**) shown as baseline-adjusted meal responses. Results are presented as mean ± SEM of log-transformed data. * – PWS *vs* obese, $P = 0{\cdot}01$.

The mechanism behind ANS changes in PWS is unclear. As postprandial insulin levels are similar between obese and PWS individuals, as well as fasting HOMA-IR (an approximate measure of insulin resistance), it is unlikely that detected differences in HRV are secondary to differences in glucose homeostasis. Further, despite evidence for an association between insulin resistance and ANS activity via postprandial thermogenesis,[**26**] this is unlikely to account for differences between the similarly insulin-resistant obese and PWS groups in the current study.

As well as being linked with cardiovascular function, measures of HRV have also been studied in the context of appetite regulation, an area of obvious interest and relevance in PWS.

Green *et al.*[**27**] found an association between eating disorders and autonomic dysfunction, while Harthoorn and Dransfield assessed the influence of sympathovagal balance on perceived satiety.[**28**]

There is evidence for the existence of a sympathetic feedback system on food intake. Rodent studies have shown a robust inverse relationship between food intake and sympathetic activity.[**29**] Further, a reduction in activity of sympathetic nerves was seen during 24- and 48-hour starvation.[**30**] Findings in human studies have been less conclusive when assessed by measurement of plasma noradrenalin levels or muscle sympathetic nerve activity (MSNA), although MSNA has been found to increase after glucose ingestion.[**31**] However, an inverse relationship has been shown between sympathetic activity and body fat.[**32**]

It is unclear whether, or to what extent, autonomic function affects appetite or satiety in PWS. PWS hyperphagia, although most likely having multifactorial influences, is strongly driven by high ghrelin levels throughout life.[**33-38**] However, our results show that sympathetic factors could also contribute. It may be that the decreased LF meal response reflects a failure of individuals with PWS to activate sympathetically mediated postprandial satiation to the same extent as control subjects.

Changes in sympathetic nervous system (SNS) activity have also been implicated in animal models of PWS. The NDN gene encoding for the protein necdin is inactive in individuals with PWS. Necdin plays a role in the terminal differentiation of neurons, and it has been shown in ndn-null mice that formation of sympathetic chain ganglia as well as axonal extension may be impaired throughout the SNS.[**39**]

In conclusion, we studied postprandial autonomic function in PWS for the first time. While we confirmed greater arterial stiffness postprandially consistent with obesity, we also suggest an impaired sympathetic meal response specific to PWS, which may contribute further to the increased cardiovascular risk and appetite dysregulation in this syndrome.

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References

1 Smith, A., Egan, J., Ridley, G. et al. (2003) Birth prevalence of Prader-Willi syndrome in Australia. Archives of Disease in Child- hood, 88, 263–264.

2 Vogels, A., Van Den Ende, J., Keymolen, K. et al. (2003) Mini- mum prevalence, birth incidence and cause of death for Prader- Willi syndrome in Flanders. European Journal of Human Genetics, 12, 238–240.

3 Burman, P., Ritzen, E.M. & Lindgren, A.C. (2001) Endocrine dysfunction in Prader-Willi syndrome: a review with special ref- erence to GH. Endocrine Reviews, 22, 787–799.

4 Goldstone, A.P., Thomas, E.L., Brynes, A.E. et al. (2001) Visceral adipose tissue and metabolic complications of obesity are reduced in Prader-Willi syndrome female adults: evidence for novel influences on body fat distribution. Journal of Clinical Endocrinology and Metabolism, 86, 4330–4338.

5 Talebizadeh, Z. & Butler, M.G. (2005) Insulin resistance and obesity-related factors in Prader-Willi syndrome: comparison with obese subjects. Clinical Genetics, 67, 230–239.

6 Greenfield, J.R., Samaras, K., Chisholm, D.J. et al. (2007) Effect of postprandial insulinemia and insulin resistance on measure- ment of arterial stiffness (augmentation

index). International Journal of Cardiology, 114, 50–56.

7 DiMario, F.J. Jr & Burleson, J.A. (2002) Cutaneous blood flow and thermoregulation in Prader-Willi syndrome patients. Pediat- ric Neurology, 26, 130–133.

8 DiMario, F.J. Jr, Dunham, B., Burleson, J.A. et al. (1994) An evaluation of autonomic nervous system function in patients with Prader-Willi syndrome. Pediatrics, 93, 76–81.

9 DiMario, F.J. Jr, Bauer, L., Volpe, J. et al. (1996) Respiratory sinus arrhythmia in patients with Prader-Willi syndrome. Journal of Child Neurology, 11, 121–125.

10 Wade, C.K., De Meersman, R.E., Angulo, M. et al. (2000) Prad- er-Willi syndrome fails to alter cardiac autonomic modulation. Clinical Autonomic Research, 10, 203–206.

11 Thayer, J.F. & Lane, R.D. (2007) The role of vagal function in the risk for cardiovascular disease and mortality. Biological Psychology, 74, 224–242.

12 Smith, G.P., Jerome, C., Cushin, B.J. et al. (1981) Abdominal vagotomy blocks the satiety effect of cholecystokinin in the rat. Science, 213, 1036–1037.

13 Gonzalez, M.F. & Deutsch, J.A. (1981) Vagotomy abolishes cues of satiety produced by gastric distension. Science, 212, 1283–1284.

14 Reidelberger, R.D., Hernandez, J., Fritzsch, B. et al. (2004) Abdominal vagal mediation of the satiety effects of CCK in rats. American Journal of Physiology: Regulatory, Integrative and Com- parative Physiology, 286, R1005–R1012.

15 Sobocki, J., Krolczyk, G., Herman, R.M. et al. (2005) Influence of vagal nerve stimulation on food intake and body weight– results of experimental studies. Journal of Physiology and Phar- macology, 56(Suppl 6), 27–33.

16 Laurent, S., Boutouyrie, P., Asmar, R. et al. (2001) Aortic stiff- ness is an independent predictor of all-cause and cardiovascular mortality in hypertensive patients. Hypertension, 37, 1236–1241.

17 Patel, S., Harmer, J.A., Loughnan, G. et al. (2007) Characteristics of cardiac and vascular structure and function in Prader-Willi syndrome. Clinical Endocrinology (Oxford), 66, 771–777.

18 Viardot, A., Sze, L., Purtell, L. et al. (2010) Prader-Willi syn- drome is associated with activation of the innate immune system independently of central adiposity and insulin resistance. Journal of Clinical Endocrinology and Metabolism, 95, 3392–3399.

19 Smith, J.C., Evans, L.M., Wilkinson, I. et al. (2002) Effects of GH replacement on endothelial function and large-artery stiffness in GH-deficient adults: a randomized, double-blind, placebo-controlled study. Clinical Endocrinology, 56, 493–501.

20 Heart rate variability. Standards of measurement, physiological interpretation, and clinical use. Task Force of the European Soci- ety of Cardiology and the North American Society of Pacing and Electrophysiology. European Heart Journal, 1996: 17, 354–381.

21 Bigger, J., Fleiss, J., Steinman, R. et al. (1992) Frequency domain measures of heart period variability and mortality after myocar- dial infarction. Circulation, 85, 164–171.

22 Grugni, G., Crino, A., Bosio, L. et al. (2008) The Italian National Survey for Prader-Willi syndrome: an epidemiologic study. American Journal of Medical Genetics A, 146, 861–872.

23 Einfeld, S.L., Kavanagh, S.J., Smith, A. et al. (2006) Mortality in Prader-Willi syndrome. American Journal of Mental Retardation, 111, 193–198.

24 Schrander-Stumpel, C.T., Curfs, L.M., Sastrowijoto, P. et al. (2004) Prader-Willi syndrome: causes of death in an interna- tional series of 27 cases. American Journal of Medical Genetics A, 124A, 333–338.

25 Stevenson, D.A., Anaya, T.M., Clayton-Smith, J. et al. (2004) Unexpected death and critical illness in Prader-Willi syndrome: report of ten individuals. American Journal of Medical Genetics A, 124A, 158–164.

26 Watanabe, T., Nomura, M., Nakayasu, K. et al. (2006) Relation- ships between thermic effect of food, insulin resistance and autonomic nervous activity. Journal of Medical Investigation, 53, 153–158.

27 Green, M.A., Hallengren, J.J., Davids, C.M. et al. (2009) An asso- ciation between eating disorder behaviors and autonomic dys- function in a nonclinical population. A pilot study. Appetite, 53, 139–142.

28 Harthoorn, L.F. & Dransfield, E. (2008) Periprandial changes of the sympathetic-parasympathetic balance related to perceived satiety in humans. European Journal of Applied Physiology, 102, 601–608.

29 Sakaguchi, T., Takahashi, M. & Bray, G.A. (1988) Diurnal changes in sympathetic activity. Relation to food intake and to insulin injected into the ventromedial or suprachiasmatic nucleus. Journal of Clinical Investigations, 82, 282–286.

30 Young, J.B. & Landsberg, L. (1977) Suppression of sympathetic nervous system during fasting. Science, 196, 1473–1475.

31 Berne, C., Fagius, J., Pollare, T. et al. (1992) The sympathetic response to euglycaemic hyperinsulinaemia. Diabetologia, 35, 873–879.

32 Bray, G.A. (2000) Reciprocal relation of food intake and sympa- thetic activity: experimental observations and clinical implica- tions. International Journal of Obesity and Related Metabolic Disorders, 24(Suppl 2), S8–S17.

33 Haqq, A.M., Grambow, S.C., Muehlbauer, M. et al. (2008) Ghre- lin concentrations in Prader–Willi syndrome (PWS) infants and children: changes during development. Clinical Endocrinology, 69, 911–920.

34 Erdie-Lalena, C.R., Holm, V.A., Kelly, P.C. et al. (2006) Ghrelin levels in young children with Prader-Willi syndrome. The Journal of Pediatrics, 149, 199–204.

35 DelParigi, A., Tschop, M., Heiman, M.L. et al. (2002) High cir- culating ghrelin: a potential cause for hyperphagia and obesity in Prader–Willi syndrome. Journal of Clinical Endocrinology and Metabolism, 87, 5461–5464.

36 Butler, M., Bittel, D. & Talebizadeh, Z. (2004) Plasma peptide YY and ghrelin levels in infants and children with Prader-Willi syndrome. Journal of Pediatric Endocrinology, 17, 1177–1184.

37 Bizzarri, C., Rigamonti, A.E., Luce, A. et al. (2010) Children with Prader-Willi syndrome exhibit more evident meal-induced responses in plasma ghrelin and peptide YY levels than obese and lean children. European Journal of Endocrinology, 162, 499–505.

38 Purtell, L., Sze, L., Loughnan, G. et al. (2011) In adults with Prader-Willi syndrome, elevated ghrelin levels are more consis- tent with hyperphagia than high PYY and GLP-1 levels. Neuro- peptides, 45, 301–307.

39 Tennese, A.A., Gee, C.B. & Wevrick, R. (2008) Loss of the Prad- er-Willi syndrome protein necdin causes defective migration, axonal outgrowth, and survival of embryonic sympathetic neurons. Developmental Dynamics, 237, 1935–1943.