View metadata, citation and similar papers at core.ac.uk
brought to you by CORE
provided by Sydney eScholarship

J.Clin Endocrinol Metab, 95(7):3392–3399, 2010

Prader-Willi Syndrome Is Associated with Activation of the Innate Immune System Independently of Central Adiposity and Insulin Resistance

Alexander Viardot, Lisa Sze, Louise Purtell, Amanda Sainsbury, Georgina Loughnan, Ellie Smith, Herbert Herzog, Katharine Steinbeck, and Lesley V. Campbell

Diabetes and Obesity Research Program (A.V., L.P., L.V.C.), Garvan Institute of Medical Research, Sydney-Darlinghurst NSW 2010, Australia; Clinics for Endocrinology, Diabetes, and Clinical Nutrition (L.S.), University Hospital Zurich, 8091 Zurich, Switzerland; Neuroscience Research Program (A.S., H.H.), Garvan Institute of Medical Research, Sydney-Darlinghurst NSW 2010, Australia; Prader-Willi Syndrome Clinic (G.L., K.S.), Metabolism and Obesity Services, Department of Endocrinology, Royal Prince Alfred Hospital, Sydney- Camperdown NSW 2050, Australia; Department of Cytogenetics (E.S.), The Children's Hospital, Westmead Clinical School, Westmead NSW 2145, Australia; University of Sydney (K.S.), Sydney NSW 2006, Australia; and Department of Endocrinology (L.V.C.), St. Vincent's Hospital, Sydney-Darlinghurst NSW 2010, Australia

Background: Subjects with Prader-Willi syndrome (PWS) have a reduced life expectancy due to cardiovascular disease. Increased systemic low-grade inflammation is postulated as a contributor, despite reported lower visceral fat mass and increased insulin sensitivity.
Objectives: Our aim was to compare inflammatory markers and arterial stiffness in PWS and adiposity-matched obese control subjects.
Design: We conducted a cross-sectional cohort study comparing 12 PWS subjects, 12 obese subjects matched for percentage body fat and central abdominal fat mass, and 10 healthy normal-weight subjects.
Main Outcome Measures: Dual-energy x-ray absorptiometry was used to assess body composition, flow cytometry to quantify activation markers on immune cells, and ELISA for measurement of C-reactive protein, adiponectin, and IL-6. Insulin resistance was estimated by homeostasis model assessment and arterial stiffness by applanation tonometry.
Results: PWS and obese subjects had similarly increased homeostasis model assessment and arterial stiffness. Nevertheless, PWS subjects showed significantly higher IL-6 (4.9 ± 1.0 *vs*. 2.5 ± 0.4 pg/ml; $P = 0.02$) and nonsignificantly higher C-reactive protein (10.5 ± 3.2 *vs*. 4.0 ± 1.0 ng/ml; $P = 0.08$). Neutrophil activation markers CD66b and CD11b were higher in PWS compared to obese subjects ($P < 0.01$), reflecting an activated innate immune system. These markers were positively related to central adiposity in lean and obese subjects ($r = 0.49$; $P < 0.05$), but not in PWS subjects.
Conclusions: PWS subjects compared to adiposity-matched obese subjects demonstrate similar insulin resistance but increased low-grade inflammation. The dissociation of inflammation and central adiposity suggests that activation of innate immunity may be either a specific genetic feature of PWS or linked to the commonly associated obstructive sleep apnea syndrome, and might offer a treatment target to reduce cardiovascular disease.

Abbreviations: AIx, Augmentation index; BMI, body mass index; CRP, C-reactive protein; CV, coefficient of variation; FFA, free fatty acid(s); HDL, high-density lipoprotein; HMW, high molecular weight; HOMA, homeostasis model assessment; HOMA-B, HOMA for -cell function; HOMA-IR, HOMA for insulin resistance; LDL, low-density lipoprotein; MFI, mean fluorescence intensity; n.s., not significant; OSAS, obstructive sleep apnea syndrome; PWS, Prader-Willi syndrome; PWSCR, PWS critical region.

Prader-Willi syndrome (PWS) is the most common known genetic cause of obesity, occurring in about one in 25,000 live births (1). It results from a lack of expression of the paternal alleles from the 15q11–q13 region on chromosome 15—the PWS critical region (PWSCR), an area that contains genes whose function is still poorly understood. The phenotype of PWS is characterized by marked hyperphagia starting in early childhood, severe obesity, GH deficiency, hypogonadism, sleep disorders, intellectual impairment, and behavioral disorders. In contrast to "simple" obesity, it has

been documented that subjects with PWS have a lower visceral fat mass relative to their weight (2), which is associated with less insulin resistance compared with weight-matched obese controls (3). However, the greater insulin sensitivity in PWS appears paradoxical with the known increased cardiovascular morbidity and mortality, which is, however, appropriate for the reported higher levels of circulating inflammation markers (4–6). This observation differs from the frequently reported finding in simple obesity that insulin resistance is strongly related to both visceral adiposity and the degree of low-grade inflammation (7). However, many studies in PWS compare subjects matched for body weight and body mass index (BMI) only, but do not correct for the difference in central adiposity. In fact, accurate body composition is infrequently assessed. Considering that visceral fat mass is a major confounder in metabolic effects and low-grade inflammation, it is difficult to draw firm conclusions from these studies as to whether higher insulin sensitivity in PWS subjects is a result of lower visceral fat mass or other factors unrelated to body composition. Therefore, in the present study, we assessed activation of the immune system in PWS compared with obese control subjects matched for age, gender, and total and central adiposity. This is the first study investigating activation of circulating immune cells together with circulating inflammation markers and assessment of arterial stiffness in relation to insulin resistance and body composition.

SUBJECTS AND METHODS

Subjects

Subjects with diagnosed PWS (n = 12) were recruited through the specialized Prader-Willi Syndrome Clinic at Royal Prince Alfred Hospital, Camperdown NSW, Australia. Obese (n = 12) and lean (n = 10) control subjects were recruited by advertisements in local newspapers and public message boards. The study was approved by the Research and Ethics Committee of St. Vincent's Hospital. All subjects gave written informed consent before starting the study. All PWS subjects had the diagnosis confirmed by cytogenetic testing (seven deletions, five uniparental disomy). Three PWS subjects and two obese control subjects had documented hypertension (blood pressure >130/80 mm Hg). There were no smokers in the PWS group, but within the obese control group, one subject was currently smoking and four had smoked previously. A positive family history of cardiovascular disease was recorded in three PWS and six obese control subjects. Three PWS subjects had type 2 diabetes (treated with metformin alone, metformin/gliclazide, and metformin/Mixtard 30/70). In addition, two obese control subjects had type 2 diabetes (treated with metformin/gliclazide/insulin glargine and metformin/sitagliptin/rosiglitazone). The two subjects on insulin treatment had fasting insulin levels close to the mean levels of their corresponding group (PWS subject, 14.6 μU/ml; obese subject, 17.1 μU/ml). Five PWS subjects and one obese control subject had diagnosed obstructive sleep apnea syndrome (OSAS). Three PWS and two obese control subjects were treated with statins, and one PWS and one obese control subject were treated with aspirin 300 mg/d. Four PWS subjects took psychotropic medication, including anticonvulsants and antidepressants, whereas two obese control subjects took antidepressants. Six of seven male PWS subjects were treated with low-dose testosterone, whereas one of four PWS females took hormone replacement therapy. No PWS subjects had received GH. All lean control subjects were healthy nonsmokers taking no medication. All subjects were studied in the fasting state, before taking any medication or insulin.

Study design

All subjects visited our Clinical Research Facility at 0800 h after a 10-h fast. All PWS subjects were supervised by parents or caregivers to ensure that the fasting instructions were followed. Height was measured by a stadiometer, and weight was measured in a hospital gown. BMI was calculated by dividing weight (in kilograms) by height (in meters) squared (kg/m2). Waist circumference was measured as the widest distance between the lower end of the ribs and the anterior superior iliac spines, and hip circumference as the widest circumference between the anterior superior iliac spines and the greater trochanters. Waist-to-hip ratio was calculated by dividing the waist circumference by the hip circumference.

Brachial blood pressure was measured by an Automatic Oscillometric Digital Blood Pressure Monitor (Omron HEM-705CP; Omron Corp., Tokyo, Japan). Two measurements were taken under

standardized conditions with the subject sitting at approximately a 45° angle in bed.
A fasting blood sample was taken, and plasma and serum were stored at −20 and −80 C and assayed at a later date, as described below. Homeostasis model assessment (HOMA) for insulin resistance (HOMA-IR) and for β-cell function (HOMA-β) was used to estimate the degree of insulin resistance and β-cell function, respectively, as described previously (8).

Body composition
Whole body dual-energy x-ray absorptiometry (Lunar DPX; GE-Lunar Corp., Madison, WI) was used to analyze body composition. As previously described (9), a central abdominal window was outlined manually extending from the upper border of L2 to the lower border of L4 and laterally to the outer margin of the rib cage. The fat in this window was measured and expressed as mass (central abdominal fat in kilograms) and as percentage of the total soft tissue content in this area. Although this window contains both intraabdominal fat and sc abdominal fat, it excludes 30% of the latter, has a relatively high intraabdominal and low sc abdominal fat content, and has been shown to be strongly inversely related to insulin sensitivity assessed by the euglycemic hyperinsulinemic clamp (9). Our group has previously demonstrated a coefficient of variation (CV) of less than 6% for central abdominal fat, based on data from 10 female subjects scanned on four separate occasions (9).

Immune cell preparation and flow cytometry analysis
Fresh whole blood was stained with fluorochrome-conjugated antibodies to various cell surface markers (CD66b, CD14, CD62L, CD11b, CD3, CD69, and CD25; BD Biosciences, San Diego, CA) using standard procedures, followed by red blood cell lysis and fixation with FACS Lyse (BD Biosciences). All analyses were performed on a BD FACSCalibur (BD Biosciences) with an excitation laser line argon (488 nm) and red diode (635 nm), and running CellQuest software (version 3.3; BD Biosciences). Data were analyzed with FlowJo software version 7 (TreeStar Inc., Ashland, OR).
For comparative quantification of activation marker expression, the mean fluorescence intensity (MFI) of the marker was divided by the MFI of the unstained control, which gives the relative MFI that can be compared among different cell subsets and subjects.

Measurement of arterial stiffness
Applanation tonometry of the radial artery was used to measure the augmentation index (AIx), a marker of arterial stiffness. A high-fidelity transducer (SyphgomoCor; AtCor Medical, Sydney, Australia) was placed over the radial artery, compressing, but not occluding, the artery against the underlying radius. The central arterial waveform was derived from the peripheral waveform using a validated transfer function (10). AIx was calculated as the ratio of the augmentation pressure and pulse pressure (×100%) (11). This technique provides intraarterial/aortic pressure measurements similar to those obtained invasively (12, 13). Previous studies have shown that AIx is inversely related to heart rate (14). Therefore, AIx was normalized to a heart rate of 75 bpm (AIx-HR75) to allow comparison between individuals. In our hands, the day to day CV for repeated fasting measurements of AIx on 4 separate days was 5.3% (15).

Biochemical variables
Plasma glucose was determined by the glucose oxidase method using a YSI glucose analyzer (model 2300 STAT PLUS 230V; YSI, Inc., Yellow Springs, OH). Serum insulin was measured by a commercial RIA (Linco, St. Charles, MO). Serum total cholesterol, high-density lipoprotein (HDL) cholesterol, and triglycerides were determined spectrophotometrically at 490 nm by enzymatic colorimetry (Roche, Basel, Switzerland). High-sensitivity C-reactive protein (CRP) was measured using a Beckman Coulter Synchron LX system Chemistry Analyser, with reagents and calibrators (Beckman Coulter Inc., Sydney, Australia). CV was 3.7% at a level of 4.9 mg/liter, and the sensitivity was 0.2 mg/liter. IL-6 was measured with a commercial high sensitive ELISA kit (R&D Systems Inc., Minneapolis, MN). CV was 6.3% at a mean level of 0.7 pg/liter. High molecular weight (HMW) adiponectin and total adiponectin were measured with a commercial multimeric EIA kit (ALPCO Diagnostics, Salem, NH). CV was 3.7 and 9.6% for total and HMW adiponectin, respectively.

Statistical analysis

Data are presented as mean ± sem for comparisons between groups. Database completion was above 99%, with one body composition, one IL-6 level, and one immune cell analysis each missing for one male PWS subject (three different subjects). Analyses were performed using Statistica 6.0 (StatSoft, Tulsa, OK). Comparisons between groups were performed using the unpaired *t* test (for normally distributed data) and the Mann Whitney-*U* test (for skewed data). Correlations between variables were expressed as Pearson's or Spearman's correlation coefficients. $P < 0.05$ was considered significant.

RESULTS

Clinical parameters

Anthropometric and biochemical characteristics of PWS, obese, and lean subjects are summarized in Table 1. Groups were matched for gender and age, and importantly, PWS and obese subjects were matched for body weight, BMI, and total body and abdominal fat mass. In PWS and matched obese subjects, HOMA-IR (an estimate of insulin resistance) and HOMA-B (an estimate of β-cell function) were significantly elevated compared with lean controls but without difference between groups. Only height and lean body mass were different between PWS and obese control subjects. PWS and obese subjects had similar low HDL-cholesterol levels compared with lean subjects, but PWS subjects differed from obese subjects by lower levels of total and low-density lipoprotein (LDL)-cholesterol. Triglyceride levels were not different between groups. Although free fatty acids (FFA) seemed to be higher in PWS compared with obese and lean controls, this difference was not statistically significant ($P = 0.28$ and 0.37, respectively). In all groups, levels of FFA were not associated with measures of obesity, central adiposity, inflammation, or insulin resistance.

TABLE 1. Mean anthropometric and metabolic measures

Variables	PWS	Obese	Lean
n (males/females)	12 (8/4)	12 (7/5)	10 (5/5)
T2D	3	2	0
Age (yr)	27.9 (2.2)	31.9 (2.5)	28.8 (1.1)
Height (cm)	154.8 (3.3)^{a,b}	167.8 (2.1)	168.7 (2.9)
Weight (kg)	92.8 (7.1)^{b}	95.9 (2.2)^{c}	60.9 (2.2)
BMI (kg/m^2)	39.0 (2.9)^{b}	34.3 (1.2)^{c}	21.4 (0.4)
Waist (cm)	114.4 (5.3)^{b}	106.3 (2.4)^{c}	72.7 (1.3)
WHR	0.93 (0.02)^{b}	0.90 (0.02)^{c}	0.79 (0.02)
Systolic BP (mm Hg)	124.1 (2.2)	126.8 (2.9)	119.5 (3.0)
Diastolic BP (mm Hg)	76.3 (2.5)^{b}	68.3 (3.1)	65.2 (2.2)
Whole body fat mass (kg)	43.3 (4.8)^{b}	40.3 (3.4)^{c}	14.6 (1.7)
Whole body fat mass (%)	49.0 (2.5)^{b}	43.1 (3.0)^{c}	25.3 (3.0)
Whole body lean mass (kg)	43.2 (2.6)^{a}	52.7 (2.6)^{c}	43.7 (2.7)
Abdominal fat mass (kg)	3.1 (0.4)^{b}	3.4 (0.3)^{c}	1.0 (0.1)
Abdominal fat mass (%)	46.6 (2.2)^{b}	46.3 (2.2)^{c}	24.8 (2.2)
Fasting glucose (mmol/liter)	4.7 (0.2)	4.9 (0.3)	4.4 (0.1)
Fasting insulin (μU/ml)	15.1 (2.0)^{b}	15.3 (1.4)^{c}	8.6 (0.6)
HOMA-IR	3.2 (0.5)^{b}	3.4 (0.4)^{c}	1.7 (0.1)
HOMA-B	60.7 (6.8)^{b}	60.2 (6.2)^{c}	35.5 (3.1)
FFA (mmol/liter)	0.55 (0.09)	0.44 (0.04)	0.46 (0.05)
Total cholesterol (mmol/liter)	3.4 (0.2)^{a,b}	4.7 (0.2)	4.2 (0.2)
HDL-cholesterol (mmol/liter)	0.9 (0.1)^{b}	1.0 (0.0)^{c}	1.4 (0.1)
LDL-cholesterol (mmol/liter)	2.1 (0.2)^{a}	3.1 (0.2)^{c}	2.5 (0.2)
Triglycerides (mmol/liter)	1.0 (0.1)	1.7 (0.4)	0.8 (0.1)

Data expressed as mean (SEM). BP, Blood pressure; T2D, type 2 diabetes; WHR, waist-to-hip ratio.

[a] PWS *vs.* obese, $P < 0.05$.

[b] PWS *vs.* lean, $P < 0.05$.

[c] Obese *vs.* lean, $P < 0.05$.

Circulating inflammation markers

Circulating levels of total and HMW adiponectin were not different across groups (Fig. 1). IL-6 levels were highest in PWS subjects (4.9 ± 1.0 pg/ml) compared with obese (2.5 ± 0.4 pg/ml; $P < 0.05$) and lean (0.8 ± 0.2 pg/ml; $P < 0.05$) subjects. CRP levels in PWS were more than double that of obese subjects (10.5 ± 3.2 *vs.* 4.0 ± 1.0 ng/ml; $P = 0.08$), and both groups had significantly higher CRP levels than lean subjects (1.1 ± 0.5 ng/ml; $P < 0.05$).

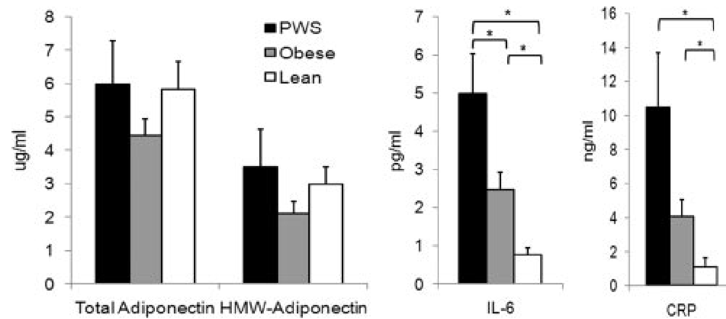

FIG. 1. Circulating levels of total and HMW adiponectin, IL-6, and CRP in subjects with PWS compared with obese and lean control subjects. *, $P < 0.05$ between groups.

Immune cell numbers and activation status

In PWS and lean subjects, a higher surface expression of CD62L was present on lymphocytes compared with obese subjects, reflecting a higher degree of lymphocyte activation in obese subjects only, but not in PWS (Fig. 2A). Expression of CD69 and CD25 was not different between groups. On granulocytes, a higher expression of CD66b and CD11b was noted in PWS compared with obese and lean subjects, whereas activation markers between lean and obese subjects were similar (Fig. 2B). On monocytes, CD11b expression was increased in both PWS and obese subjects, compared with lean subjects. PWS showed a weak trend toward increased CD11b expression compared with obese subjects ($P = 0.1$). Obese subjects demonstrated decreased levels of CD62L expression compared with lean subjects (Fig. 2C), reflecting increased cell activation.

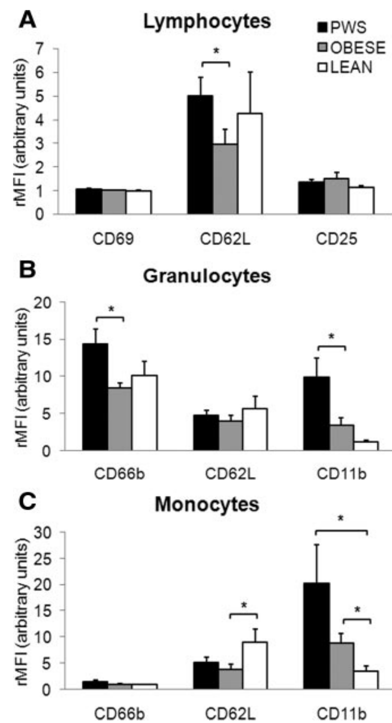

FIG. 2. Expression of surface activation markers CD69, CD62L, and CD25 on lymphocytes (A), and CD66b, CD62L, and CD11b on granulocytes (B) and monocytes (C) in subjects with PWS (*black bars*) compared with obese (*gray bars*) and lean (*white bars*) control subjects. *, $P < 0.05$ between groups. rMFI, Relative MFI.

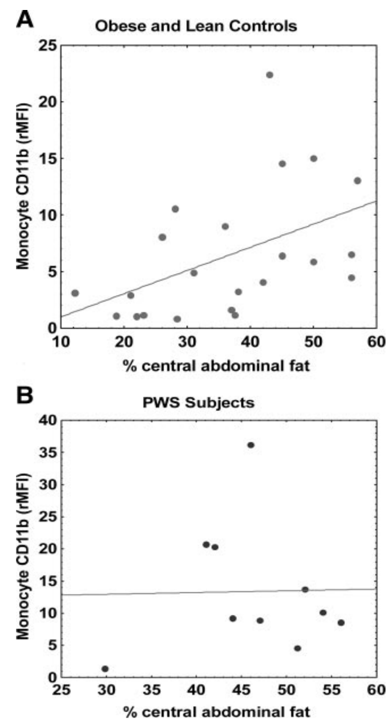

FIG. 3. Central adiposity predicts activation status of innate immune cells (monocyte expression of surface activation marker CD11b) in combined obese and lean control groups (A; $r = 0.57$; $P < 0.05$), but not in the PWS group (B; $r = -0.14$; $P =$ n.s.). rMFI, Relative MFI.

In lean and obese subjects, abdominal fat mass (percentage of central abdominal fat) was closely related to granulocyte expression of CD11b (r = 0.47; $P < 0.05$) as well as monocyte expression of CD11b (r = 0.57; $P < 0.05$; Fig. 3A) and CD62L (r = −0.50; $P < 0.05$). However, these associations were completely absent in the PWS group: granulocyte CD11b [r = 0.1; *P* = not significant (n.s.)], monocyte CD11b (r = −0.14; *P* = n.s.; Fig. 3B), and monocyte CD62L (r = −0.42; *P* = n.s.). Activation markers of lymphocytes did not correlate with measures of central adiposity.

Most immune cell activation markers related closely to circulating inflammation markers: IL-6 levels correlated positively with lymphocyte CD25 (r = 0.43; $P < 0.05$), granulocyte CD66b and CD11b (r = 0.42, $P < 0.05$; and r = 0.65, $P < 0.05$, respectively), and monocyte CD11b (r = 0.62; $P < 0.05$). Similarly, CRP levels correlated positively with lymphocyte CD25 ($P = 0.46$; $P < 0.05$), granulocyte CD11b ($P = 0.48$; $P < 0.05$), and monocyte CD11b (r = 0.54; $P < 0.05$).

Arterial stiffness

Arterial stiffness (AIx) was elevated in PWS (17.6 ± 3.0) and obese subjects (14.5 ± 2.7) compared with lean subjects (0.6 ± 3.8; $P < 0.05$) (Fig. 4A). The difference in AIx between PWS and obese subject did not reach statistical significance ($P = 0.1$). Across the whole cohort, AIx was highly associated with levels of IL-6 (r = 0.64; $P < 0.05$), CRP (r = 0.52; $P < 0.05$), monocyte CD11b expression (r = 0.49; $P < 0.05$), and abdominal fat mass (r = 0.65; $P < 0.05$) (Fig. 4B). When PWS subjects were analyzed separately, the above correlations remained similar; however, they lost statistical significance due to the small cohort size (AIx *vs.* IL-6, r = 0.42, $P = 0.1$; AIx *vs.* CRP, r = 0.33, $P = 0.3$; AIx *vs.* monocyte CD11b, r = 0.19, $P = 0.57$; AIx *vs.* abdominal fat mass, r = 0.59, $P = 0.055$).

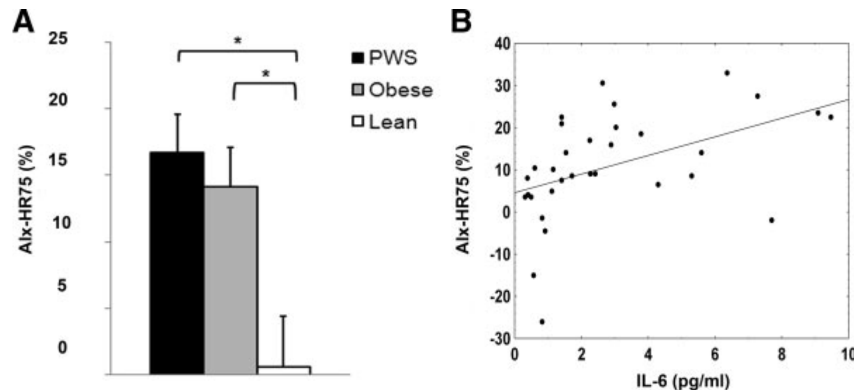

FIG. 4. Arterial stiffness (AIx) in subjects with PWS (*black bar*) compared with obese (*gray bar*) and lean (*white bar*) control subjects (A), and its association with increased levels of IL-6 (B; r = 0.64; $P < 0.05$). *, $P < 0.05$ between groups.

Effects of OSAS and statin treatment on inflammation

Five of 12 PWS subjects and one of 12 obese control subjects had diagnosed OSAS. This syndrome has been reported to be independently associated with systemic inflammation; however, the underlying mechanism is still poorly understood. A subgroup analysis of our PWS subjects with and without OSAS revealed that OSAS was associated with a trend toward higher CRP levels (17.3 *vs.* 5.6 ng/ml; $P = 0.07$), higher CD62L expression on lymphocytes (70.6 *vs.* 36.2 arbitrary units; $P = 0.017$), as well as higher monocyte expression of CD66b (2.4 *vs.* 1.1; $P = 0.017$). Monocyte expression of CD11b was not different in subjects with and without OSAS (30.5 *vs.* 14.3; $P = 0.32$). Importantly, PWS subjects with OSAS had a trend toward an increased abdominal fat mass (3.8 *vs.* 2.6 kg; $P = 0.17$).

Three PWS and two obese subjects were on statin treatment, which is known to modulate systemic inflammation. Within the PWS group, subjects taking statins had higher FFA levels (0.85 *vs.* 0.43 mmol/liter; $P = 0.017$) and a trend toward lower lymphocyte CD62L expression (27.9 *vs.* 59.4; $P = 0.055$). Lipid levels were not different between PWS subjects with and without statins. Among

obese subjects, those on statin treatment had lower HDL levels (0.8 *vs.* 1.0 mmol/liter; $P = 0.04$), LDL levels (1.6 *vs.* 3.3 mmol/liter; $P = 0.03$), and triglycerides (1.2 *vs.* 4.4 mmol/liter; $P = 0.001$). Furthermore, total and HMW adiponectin levels were lower in subjects taking statins (1.4 *vs.* 4.9 µg/ml, $P = 0.004$; and 0.3 *vs.* 2.3 µg/ml, $P = 0.003$, respectively). Other inflammation markers or immune cell activation markers were not different in subjects under statin treatment.

DISCUSSION

PWS is associated with an increased risk of cardiovascular morbidity and mortality, discrepant with previous reports of lower visceral fat mass and higher insulin sensitivity compared with nonsyndromic obesity (3, 16, 17). To assess the contribution of chronic inflammation to the cardiovascular risk in PWS, it is most important to match the groups compared for total and visceral adiposity to eliminate the impact of total and visceral fat mass as major confounders. To the best of our knowledge, this is the first study to carefully match PWS and obese control subjects for visceral and total adiposity, in addition to age and gender.

In our matched PWS and obese control subjects, we observed similar insulin and glucose levels and estimated insulin resistance (HOMA-IR) and insulin secretion (HOMA-B). Nevertheless, we observed higher levels of IL-6 and a trend toward elevated CRP levels in PWS subjects. Furthermore, our results suggest that PWS is associated with increased activation of the innate immune system compared with simple obesity. Across all groups, most immune cell activation markers were positively related to levels of circulating inflammation markers. It has been previously reported that "simple" obesity is associated with increased expression of cell surface activation markers on neutrophils, monocytes, and T lymphocytes; and up-regulation of CD66b, CD11b, CD69, and CD25 and down-regulation of CD62L is thought to reflect increased immune cell activation (18–20). Interestingly, the observed down-regulation of CD62L on lymphocytes was only seen in obese subjects, not in PWS subjects, suggesting that in contrast to obese subjects, the observed cell activation is specifically seen in the innate immune system, excluding cells of the adaptive immune system (lymphocytes). Our results suggest that in PWS there is a disruption of the association between low-grade inflammation and central adiposity, different from that reported in obese subjects (7). Intriguingly, we observed in our PWS subjects that total and HMW adiponectin levels were similar compared with obese and lean controls. This is in contrast with previous reports (21–23) where PWS subjects had higher adiponectin levels in association with lower visceral fat mass and preserved insulin sensitivity. Our PWS subjects had similarly increased central adiposity and insulin resistance to obese control subjects, and finding similar adiponectin levels is not a surprise. However, the high adiponectin levels are in contradiction to the increased immune activation, inflammation, and increased cardiovascular morbidity in PWS because protective antiinflammatory and antiatherogenic effects of adiponectin have been reported (24).

Both IL-6 and CRP levels were highly correlated with measures of total and visceral adiposity, as well as HOMA-IR. Similarly, AIx, a marker of arterial stiffness, was closely related to IL-6, high-sensitivity CRP, and monocyte activation marker CD11b, as illustrated in Fig. 4. AIx was also related to measures of total and visceral adiposity, but not HOMA-IR. Our data suggest that immune activation is directly associated with endothelial dysfunction, increasing arterial stiffness and contributing to the elevated risk for cardiovascular disease in PWS. There are only sparse data on endothelial dysfunction in PWS, and only one study previously assessed arterial stiffness, flow-mediated vasodilatation, and venous occlusion plethysmography, but only comparing PWS to lean subjects (5). The venous occlusion plethysmography, but not the AIx, was found to be abnormal in PWS. Our study adds new information about similarly impaired endothelial function in PWS and adiposity-matched obese subjects compared with healthy lean control subjects.

Apart from elevated inflammatory markers, no other major cardiovascular risk factors differed in our PWS and obese control group. There was no difference in blood pressure, AIx, or smoking status. The lipid profile showed a lower total and LDL-cholesterol in PWS compared with obese subjects, an observation consistent with previous reports (3). Circulating FFA, which have been reported to be positively related to measures of obesity and systemic inflammation in obesity (25), were not different between groups, consistent with previous reports (26). This suggests that

FFA are unlikely to significantly contribute to the observed increased activation of the innate immune system. Statin treatment seemed to affect lipid levels in obese subjects only, whereas in PWS, statin treatment was not associated with altered lipid levels except for higher levels of FFA. This suggests that the rather beneficial lipid profile in PWS is not due to increased use of statin treatment in this group. Furthermore, statin treatment did not seem to significantly affect markers of inflammation or immune cell activation, except for the documented decrease in lymphocyte CD62L in PWS subjects, which suggests an antiinflammatory effect on this immune cell subset. However, it is important to mention that this study was not designed to investigate the effects of statins on immune activation, and prospective controlled studies are needed to clarify statin effects on inflammation, immune activation, as well as cardiovascular morbidity and mortality in subjects with PWS.

Importantly, some frequently diagnosed comorbidities could affect systemic inflammation in our PWS subjects. First, obstructive sleep apnea, which was diagnosed in five PWS subjects in contrast to only one obese control, is a syndrome that has itself been linked to increased low-grade inflammation (27, 28). Indeed, our subgroup analysis suggests that PWS subjects with OSAS tend to have higher levels of CRP and immune cell activation, although many of these differences are not or are only borderline significant. Second, subjects with PWS are known to be GH deficient, which is also reported to be associated with increased systemic inflammation (29–31) and endothelial dysfunction (32–34). None of our PWS subjects had been treated with GH before or at the time of study. Third, hypogonadism is a known feature of PWS and has been shown to be associated with increased systemic inflammation (35). Although most of our PWS subjects were receiving hormone replacement therapy, the doses were generally low, and we cannot exclude the possibility that mild hypogonadism somehow accounts for the observed increase in chronic inflammation.

The PWSCR on chromosome 15 is complex genetically, subject to strict genomic imprinting via an imprinting center. Control of the imprinting center is by DNA methylation and epigenetic mechanisms. The PWSCR contains many genes whose function is not well understood and harbors many small noncoding RNAs that play an important role in translational regulation of gene expression (36). Due to the absent or altered expression of a wide range of genes in the PWSCR, activation of the innate immune system could well be part of the genetic phenotype. Epigenetic modulations are well-recognized regulators of the immune response, and the largest part of the interindividual variances in immune response to pathogens is thought to be due to epigenetic modifications (37). Disturbed activity of the autonomic nervous system has been suggested to play a role in PWS (38), and a decreased parasympathetic activity could play a role in activation of the innate immune system, secondary to genetic dysregulation of the autonomic nervous system. Disturbance of this complex system could also play a role in the pathogenesis of OSAS, and it could be difficult to separate direct and indirect effects on regulation of the immune system.

It should be noted that studying subjects with PWS is associated with many challenges that restrict the number of study participants investigated. These challenges include difficult cannulation, subject lack of cooperation, and availability of willing family members or caregivers to take part in the research. However, a major strength of the present study is that we were able to use optimal methods to assess body composition that were previously validated against insulin sensitivity in obesity, which also allowed us to compare PWS subjects to obese subjects closely matched for both central and total adiposity as well as gender. In previous studies, apparent differences in inflammatory markers could be due to confounding differences in adiposity (total or central) or by gender mismatching, which itself influences total adiposity and fat distribution. It is important to note that there are no validation data on estimating central abdominal fat mass in PWS, and we can't exclude an overestimation in PWS due to increased sc fat mass in this area. However, the same method has been used in previous PWS studies, where it commonly revealed a lower abdominal fat mass, in association with low insulin levels. The consistent association of high insulin levels and high abdominal fat mass to a similar degree in both PWS and matched obese subjects in this study suggests that the dual-energy x-ray absorptiometry methodology consistently provides a reasonable estimate for central obesity.

In summary, this study reports evidence for an overactivation of the innate immune system in PWS, independent of central adiposity and insulin resistance. Increased low-grade inflammation is associated with increased arterial stiffness, a recognized marker for increased cardiovascular risk.

Therefore, we suggest that increased chronic low-grade inflammation, caused either by genetic and/or epigenetic abnormalities of PWS or by mechanisms linked to the commonly associated OSAS, may be a potential treatment target in the clinical setting to improve cardiovascular morbidity and mortality in PWS.

ACKNOWLEDMENTS

Our thanks go to the staff of the Garvan Clinical Research Facility and to the volunteers who participated in the study.

This study was funded by a research grant established by supporters and families of children with Prader-Willi syndrome. A.V. is the recipient of a GlaxoSmithKline Don Chisholm Diabetes Research Fellowship, L.S. is the recipient of a grant for "prospective researchers" from the Swiss National Science Foundation. A.S. and H.H. are supported by fellowships from the National Health and Medical Research Council of Australia.

REFERENCES

1. **Whittington JE, Holland AJ, Webb T, Butler J, Clarke D, Boer H** 2001 Population prevalence and estimated birth incidence and mor- tality rate for people with Prader-Willi syndrome in one UK Health Region. J Med Genet 38:792–798

2. **Goldstone AP, Thomas EL, Brynes AE, Bell JD, Frost G, Saeed N, Hajnal JV, Howard JK, Holland A, Bloom SR** 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. J Clin Endocrinol Metab 86:4330–4338

3. **Talebizadeh Z, Butler MG** 2005 Insulin resistance and obesity-re- lated factors in Prader-Willi syndrome: comparison with obese subjects. Clin Genet 67:230 –239

4. **Caixas A, Gimenez-Palop O, Broch M, Vilardell C, Megía A, Simo´ n I, Gimenez-P rez G, Mauricio D, Vendrell J, Richart C, Gonza´ lez- Clemente JM** 2008 Adult subjects with Prader-Willi syndrome show more low-grade systemic inflammation than matched obese sub- jects. J Endocrinol Invest 31:169 –175

5. **Patel S, Harmer JA, Loughnan G, Skilton MR, Steinbeck K, Celermajer DS** 2007 Characteristics of cardiac and vascular structure and function in Prader-Willi syndrome. Clin Endocrinol (Oxf) 66:771–777

6. **Butler MG, Bittel DC, Kibiryeva N, Garg U** 2006 C-Reactive protein levels in subjects with Prader-Willi syndrome and obesity. Genet Med 8:243–248

7. **Greenfield JR, Samaras K, Jenkins AB, Kelly PJ, Spector TD, Gallimore JR, Pepys MB, Campbell LV** 2004 Obesity is an important determinant of baseline serum C-reactive protein concentration in monozygotic twins, independent of genetic influences. Circulation 109:3022–3028

8. **Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC** 1985 Homeostasis model assessment: insulin resistance and -cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia 28:412– 419

9. **Carey DG, Jenkins AB, Campbell LV, Freund J, Chisholm DJ** 1996 Abdominal fat and insulin resistance in normal and overweight women: direct measurements reveal a strong

relationship in subjects at both low and high risk of NIDDM. Diabetes 45:633– 638

10. **Karamanoglu M, O'Rourke MF, Avolio AP, Kelly RP** 1993 An analysis of the relationship between central aortic and peripheral upper limb pressure waves in man. Eur Heart J 14:160 –167

11. **Cameron JD, McGrath BP, Dart AM** 1998 Use of radial artery applanation tonometry and a generalized transfer function to determine aortic pressure augmentation in subjects with treated hypertension. J Am Coll Cardiol 32:1214–1220

12. **Hayward CS, Kraidly M, Webb CM, Collins P** 2002 Assessment of endothelial function using peripheral waveform analysis: a clinical application. J Am Coll Cardiol 40:521–528

13. **Kelly R, Hayward C, Avolio A, O'Rourke M** 1989 Noninvasive determination of age-related changes in the human arterial pulse. Circulation 80:1652–1659

14. **Wilkinson IB, MacCallum H, Flint L, Cockcroft JR, Newby DE, Webb DJ** 2000 The influence of heart rate on augmentation index and central arterial pressure in humans. J Physiol 525:263–270

15. **Greenfield JR, Samaras K, Hayward CS, Chisholm DJ, Campbell LV** 2005 Beneficial postprandial effect of a small amount of alcohol on diabetes and cardiovascular risk factors: modification by insulin resistance. J Clin Endocrinol Metab 90:661–672

16. **Goldstone AP, Brynes AE, Thomas EL, Bell JD, Frost G, Holland A, Ghatei MA, Bloom SR** 2002 Resting metabolic rate, plasma leptin concentrations, leptin receptor expression, and adipose tissue measured by whole-body magnetic resonance imaging in women with Prader-Willi syndrome. Am J Clin Nutr 75:468–475

17. **Marzullo P, Marcassa C, Campini R, Eleuteri E, Minocci A, Priano L, Temporelli P, Sartorio A, Vettor R, Liuzzi A, Grugni G** 2005 The impact of growth hormone/insulin-like growth factor-I axis and nocturnal breathing disorders on cardiovascular features of adult patients with Prader-Willi syndrome. J Clin Endocrinol Metab 90: 5639 –5646

18. **Cottam DR, Schaefer PA, Fahmy D, Shaftan GW, Angus LD** 2002 The effect of obesity on neutrophil Fc receptors and adhesion molecules (CD16, CD11b, CD62L). Obes Surg 12:230 –235

19. **Stentz FB, Kitabchi AE** 2005 Hyperglycemia-induced activation of human T-lymphocytes with de novo emergence of insulin receptors and generation of reactive oxygen species. Biochem Biophys Res Commun 335:491–495

20. **van Oostrom AJ, van Wijk JP, Sijmonsma TP, Rabelink TJ, Castro Cabezas M** 2004 Increased expression of activation markers on monocytes and neutrophils in type 2 diabetes. Neth J Med 62:320 –325

21. **Haqq AM, Muehlbauer M, Svetkey LP, Newgard CB, Purnell JQ, Grambow SC, Freemark MS** 2007 Altered distribution of adiponec- tin isoforms in children with Prader-Willi syndrome (PWS): associ- ation with insulin sensitivity and circulating

satiety peptide hormones. Clin Endocrinol (Oxf) 67:944 –951

22. **Kennedy L, Bittel DC, Kibiryeva N, Kalra SP, Torto R, Butler MG** 2006 Circulating adiponectin levels, body composition and obesity- related variables in Prader-Willi syndrome: comparison with obese subjects. Int J Obes (Lond) 30:382–387

23. **Hoybye C, Bruun JM, Richelsen B, Flyvbjerg A, Frystyk J** 2004 Serum adiponectin levels in adults with Prader-Willi syndrome are independent of anthropometrical parameters and do not change with GH treatment. Eur J Endocrinol 151:457– 461

24. **Ziemke F, Mantzoros CS** 2010 Adiponectin in insulin resistance: lessons from translational research. Am J Clin Nutr 91:258S–261S .

25. **Ghanim H, Aljada A, Hofmeyer D, Syed T, Mohanty P, Dandona P** 2004 Circulating mononuclear cells in the obese are in a proinflammatory state. Circulation 110:1564 –1571.

26. **Butler MG, Carlson MG, Schmidt DE, Feurer ID, Thompson T** 2000 Plasma cholecystokinin levels in Prader-Willi syndrome and obese subjects. Am J Med Genet 95:67–70.

27. **Chorostowska-Wynimko J, Radomska D, Plywaczewski R, Jonczak L, Stepniewska A, Gorecka D, Skopinska-Rozewska E** 2005 Disturbed angiogenic activity in sera from obstructive sleep apnea patients. J Physiol Pharmacol 56(Suppl 4):71–77

28. **Minoguchi K, Yokoe T, Tazaki T, Minoguchi H, Tanaka A, Oda N, Okada S, Ohta S, Naito H, Adachi M** 2005 Increased carotid intima media thickness and serum inflammatory markers in obstructive sleep apnea. Am J Respir Crit Care Med 172:625– 630

29. **Heald AH, Anderson SG, Ivison F, Laing I, Gibson JM, Cruickshank K** 2003 C-Reactive protein and the insulin-like growth factor (IGF)- system in relation to risk of cardiovascular disease in different ethnic groups. Atherosclerosis 170:79 – 86

30. **Kaushal K, Heald AH, Siddals KW, Sandhu MS, Dunger DB, Gibson JM, Wareham NJ** 2004 The impact of abnormalities in IGF and inflammatory systems on the metabolic syndrome. Diabetes Care 27:2682–2688

31. **Twickler TB, Dallinga-Thie GM, Visseren FL, de Vries WR, Erkelens DW, Koppeschaar HP** 2003 Induction of postprandial inflammatory response in adult onset growth hormone deficiency is related to plasma remnant-like particle-cholesterol concentration. J Clin Endocrinol Metab 88:1228 –1233

32. **Hana V, Prazny M, Marek J, Skrha J, Justova V** 2002 Reduced microvascular perfusion and reactivity in adult GH deficient patients is restored by GH replacement. Eur J Endocrinol 147:333–337

33. **Soares DV, Spina LD, de Lima Oliveira Brasil RR, da Silva EM, Lobo PM, Salles E, Coeli CM, Conceiçao FL, Vaisman M** 2005 Carotid artery intima-media thickness and lipid profile in adults with growth hormone deficiency after long-term growth hormone replacement. Metabolism 54:321–329

34. **Lanes R** 2008 Endothelial dysfunction in growth hormone deficiency. J Pediatr Endocrinol

Metab 21:319 –321

35. **Laaksonen DE, Niskanen L, Punnonen K, Nyyssonen K, Tuomainen TP, Salonen R, Rauramaa R, Salonen JT** 2003 Sex hormones, inflammation and the metabolic syndrome: a population-based study. Eur J Endocrinol 149:601– 608

36. **Sahoo T, del Gaudio D, German JR, Shinawi M, Peters SU, Person RE, Garnica A, Cheung SW, Beaudet AL** 2008 Prader-Willi phenotype caused by paternal deficiency for the HBII-85 C/D box small nucleolar RNA cluster. Nat Genet 40:719 –721

37. **Wilson AG** 2008 Epigenetic regulation of gene expression in the inflammatory response and relevance to common diseases. J Periodontol 79:1514 –1519

38. **Kreier F, Fliers E, Voshol PJ, Van Eden CG, Havekes LM, Kalsbeek A, Van Heijningen CL, Sluiter AA, Mettenleiter TC, Romijn JA, Sauerwein HP, Buijs RM** 2002 Selective parasympathetic innervation of subcutaneous and intra-abdominal fat—functional implications. J Clin Invest 110:1243–1250