SUPPLEMENTAL MATERIALS

THE COMPREHENSIVE IMPACT ON HUMAN BODY INDUCED BY WITHDRAWAL OF GROWTH HORMONE EXCESS

Supplemental Methods

Oral glucose tolerance test (OGTT)
At each visit, standard 75g OGTT was performed. Serum specimens collected at 0, 30, 60, 120, and 180 minutes were used to measure concomitant growth hormone (GH), glucose, insulin and c-peptide levels. Diabetes mellitus, impaired fasting glucose (IFG) and impaired glucose tolerance (IGT) were defined according to the ADA 2010 criteria. Indices of islet function, insulin resistance and insulin sensitivity were calculated as previously defined. HOMA-IR was calculated as previously defined.

Biochemical measurements
GH level was measured by chemiluminescent immunometric assay (ImmuliteTM, Siemens, USA). Insulin-like growth factor-I (IGF-I) level was measured with chemiluminescence immunoassay (Immulite2000, Siemens, USA). IGF-I level was adjusted by age in Chinese population. IGF-I index was calculated as IGF-I value divided by the upper limit of reference range for IGF-I. Adrenocorticotropic-hormone (ACTH) was measured with immunofluorimetric assay (AutoDelfia kit, Wallac, Turku, Finland). Thyroid hormones, insulin and c-peptide were measured by chemiluminescence immunoassay (ADVIA Centaur XP, Siemens, USA). Other hormones (luteinizing hormone [LH], follicle-stimulating hormone [FSH], progesterone, testosterone, estrogen, cortisol and prolactin [PRL]) were measured by electrochemiluminescence system (Elecsys modular analytics E-170, Roche, USA). Men with a morning total testosterone level of <8.0 nmol/L were considered hypogonadal. In females, gonadal dysfunction was diagnosed in premenopausal females if they presented with menstrual disturbances and in postmenopausal females if the FSH was inappropriately low (<35 IU/L). Glycated hemoglobin (HbA1c) was detected with high performance liquid chromatography (Tosoh HLC-723 G8 HPLC Analyzer, Japan); hepatic and renal function, electrolyte, glucose and lipid profiles including total cholesterol (TC), high-density lipoprotein (HDL), low density lipoprotein (LDL), triglycerides, apolipoprotein A1(Apo A1), Apo B and lipoprotein (a)(lip[a]) levels were measured by HITACHI 7600 Biochemical Analyzer (Tokyo, Japan); C-reactive protein (CRP) was measured by particle-enhanced immunonephelometry (BN II system, Siemens, USA). Blood routine tests were examined by XN-2000 hematology analyzer (Sysmex, Japan). N-Mid osteocalcin, procollagen type one N-terminal propeptide (P1NP), β-isomer of C-terminal telopeptide of type I collagen (β-CTX) were measured by electrochemiluminescence immunoassay using a cobas e411 automated immunoassay system (Roche Co., Mannheim, Germany). Urine routine tests were performed by AX-4280 urine dry chemistry analyzer (Akaray, Japan). 24-hour urine free cortisol (UFC) was measured with electrochemiluminescence system (Elecsys modular analytics E-170, Roche, USA).

Evaluation of pituitary tumor size
All patients underwent pituitary magnetic resonance imaging (MRI) examinations before and after surgery. Maximal tumor diameter was taken as the largest measurement in the coronal, axial, or sagittal planes. Tumor size was classified in three categories according to maximum tumor diameter: microadenoma (<10 mm); macroadenomas (>10 mm); and giant adenoma (>40 mm). Cavernous sinus invasion was categorized according to the Knosp classification based on coronal T1-weighted contrasted imaging.

24-hour ambulatory blood pressure monitoring (ABPM)
The blood pressure profiles were evaluated by 24-hour ABPM. SCHILLER BR-102 plus (Schiller AG, Bear, Switzerland) is a menu-guided user-programmable device to accomplish individual measurements. The monitors were programmed to record measurements at 15-minute intervals during the daytime (from six am to 10 pm) and 20-minute during night-time (from 10 pm to six am). Mean daytime, nocturnal and 24-hour blood pressure values were obtained. Daytime was considered to be from 1000 hour to 1800 hour, and nocturnal from 0000 hour to 0600 hour, to exclude the transition periods when BP often undergoes sudden and marked variations. Normal ambulatory SBP/DBP values were defined as <135/85 mmHg for daytime, <120/70 mmHg for night-time and <130/80 mmHg for 24-hour measurements. Systolic/diastolic thresholds for ambulatory hypertension were defined as 140/90 mmHg for daytime, 125/75 mmHg for night-time and 135/85 mmHg for 24-hour according to the current guidelines.

**Echocardiography**

Trans-thoracic M-Mode and two-dimensional color Doppler echocardiographic studies were performed with equipment IE33 [Philips Medical Systems, Andover, MA, USA; 5-1 (one to five MHz) probe for 2D imaging]. According to the recommendations from the American Society of Echocardiography, patients were examined in the left lateral recumbent position after a 10-minute resting period; records were made by trained and certified sonographers during three to four consecutive cardiac cycles. Total ejection isovolume (Tei) index was defined and calculated based on previous report.

**Pulmonary function measurement**

The pulmonary function was performed with the patient seated comfortably using the Master Screen Body (Jaeger, Germany). The procedures and tests were well explained and performed following the guides of a trained and skilled technician. Data including tidal volume (VT), total lung capacity (TLC), residue volume (RV), expiratory reserve volume (ERV), minute ventilation (MV), forced vital capacity (FVC), forced expiratory volume (FEV), FEV1/FVC, peak expiratory flow (PEF), diffusion capacity for carbon monoxide (DLCO), maximum inspiratory pressure (MIP), maximum expiratory pressure (MEP) were collected. Predicted pulmonary function data were measured according to the maximal-effort expiratory spirogram. Four patients were smokers including one female patient. To evaluate pulmonary function, the percentages of predicted values were collected. Large lung was defined as total lung capacity (TLC) greater than predicted (above 95% confidence limits).

**Polysomnography (PSG)**

Each participant was scheduled to complete all-night attended PSG recordings by a digital system (Compumedics S-Series Sleep System, Compumedics Ltd, Melbourne, Australia). All PSG scoring was performed before operation and at post-operation visits of one month, three months and six months, while the 12-month follow-up was optional. The onset and offset record times were determined by each participant’s habitual bedtime and uptime, with the time period approximately from 22:00 to 06:00. Parameters were calculated based on previous report. Secure data were collected: total sleep time (TST), total wake time, sleep efficacy, number of awakenings, apnea-hypopnea index (AHI), and oxygen saturation. Apnea was defined when breathing cessation for 10 seconds or longer, hypopnea was recorded when airflow was below 50% for at least 10 seconds, accompanied by oxyhemoglobin desaturation or arousal from sleep. The type of sleep apnea syndrome (SAS) was divided into obstructive (OSAS), central (CSAS) or mixed (OSAS+CSAS). AHI (number of apnea and hypopnea episodes per hour) was used to evaluate OSA status, as values of 5–15, 15–30, and more than 30 was define as mild, moderate, and severe OSAS, respectively.

**Bone mineral density (BMD)**

BMD was evaluated by dual-energy X-ray absorptiometry using a Discovery W (Hologic ASY-00409, Bedford, MA, software version 12.3). BMD was measured at the first to fourth lumbar vertebrae and left hip (femoral neck, trochanter, inter, Ward’s). Area and bone mineral content were recorded to calculate absolute values.
BMD was expressed in absolute values (g/cm²) and as standard deviation (SD) from the peak bone mass (T-score) and from the expected BMD for the age-matched population (Z-score).16

**Computer tomography (CT) scanning and 3-dimension model reconstruction**

The upper airway and abdomen thin slice CT scanning were performed by CT scanner Brilliance iCT 256 Slice (Philips, USA) or Somato Sensation 64 (Siemens, Germany), using a 1.5mm thickness. We transferred the adopted DICOM data sets into Osirix to generate a three-dimension model. Radiologist with 10 years CT experience manually traced the contours of liver, renal (excluding renal pedicle and pelvis), splenic (excluding hilum of spleen), pancreatic and adrenal sections, while the software automatically calculated the corresponding organ volumes. The upper airway was extracted automatically by the program after optimal threshold for the air–soft-tissue interface was determined. The following arbitrary reproducible anatomic limits for the upper airway volume were adopted: cephalad (the hard palate plane), caudad (the glottis), ventral (the junction of the superior adenoid tissue and the nasopharynx), dorsal (the posterior pharyngeal wall), and lateral (the right and left lateral pharyngeal walls).17 Patients were required to wear lead shields for other parts of the body to prevent radiation exposure.

**Anthropometric and biochemical measurements**

Body mass index (BMI) was calculated as the weight in kilograms divided by the square of the height in meters. Anthropometric examinations were performed in the supine position by Vernier caliper. Morphological width of the nose was defined as the distance between lateral points where the nasal blade extends farthest out. Upper lip height is the vertical distance from oral fissure to upper vermilion border in the middle line, and to lower vermilion border for lower lip height. Finger circumferences and foot lengths were measured using an inelastic measuring tape. Hand volume was measured by drainage.

Modification of diet in renal disease (MDRD) equation recalibrated for Chinese was used to estimate eGFR expressed in ml/min/1.73 m²: eGFR = 186 * [SCR *0.011]^{1.154} * [age]^{-0.203} * [0.742 if female] *1.233, where SCR is serum creatinine expressed as mmol/L and 1.233 is the coefficient for Chinese.19
SUPPLEMENTARY RESULTS

Basic characteristics of the cohort

Forty-eight patients with acromegaly were initially recruited in this study. At one year visit, 24 patients were considered to be surgically cured, while the other 24 patients still had active disease. In the cured group, there were three (12.5%) microadenomas and 21 (87.5%) macroadenomas. In the non-remission group, 1 (4.17%) patient presented with microadenoma, 20 (83.3%) with macroadenomas and three (12.5%) with giant adenomas. Remission was thus established in 75% of microadenomas, 51.22% of macroadenomas and none in giant adenomas. The non-remission group received adjuvant treatment and was excluded from the study.

Therefore, 24 patients were included in the final analysis. At enrollment, the mean age was 43.79±12.22 years (range19-61), with a slight male preponderance (17/7). Median time from the onset of symptoms to diagnosis was 4.5 years (rang one to 20 years). Median tumor diameter was 0.0185 m (0.0113-0.0208 m). Postoperative cerebrospinal fluid leaks occurred in 5 (20.83%) patients and transient diabetes insipidus in two (8.33%) patients. Two patients were diagnosed with panhypopitutarsim and were on replacement medication. Pathological examinations and immunohistochemical staining confirmed typical GH-producing somatotroph adenomas in all patients.

Compared with the baseline, BMI slightly declined at one week postoperatively (26.47± 2.96 vs 25.78± 2.83 kg/m², P=0.03), but it went back to baseline level at later visits.

TSH and FT₄ levels showed no change over the entire follow-up period (Supplementa Figure 1, A and B). FT₃ got lower at one week (4.40±0.95 vs 2.93±0.71 pmol/L, P<0.001) while went back to the pre-surgical level at one month, suggesting the transient existence of low T₃ syndrome (Supplementa Figure 1C).

Both ACTH and morning cortisol were unchanged from the pre-surgical visit to the one year follow-up. However, compared with the baseline, 24-hour urinary free cortisol (UFC) at both six months and 12 months decreased, which was consistent with previous reports showing that patients with active acromegaly had higher UFC than controls. Testosterone in male patients decreased at one week (7.31±3.12 vs 3.68±3.54 nmol/L, P=0.001), while rose to 12.72±7.78 nmol/L at three months (P<0.001 compared to one week) (Supplementa Figure 1D). Among the 17 male patients, ten (58.82%) had low testosterone at baseline while at one year, eight (80%) of them recovered normal gonadal function. The change pattern in DHEA was consistent with the testosterone. In females, gonadal dysfunction was diagnosed in premenopausal females if the patient presented with menstrual disturbances and in postmenopausal females if the FSH was inappropriately low (<3 IU/L). Of the seven female patients, three presented with hypogonadism at baseline. Among them, two were in menopause and one patient had menstrual disturbances. The former two revealed normal gonadal function while the latter one recovered regular cycles at one month after the surgery.

All patients demonstrated a reduction in PRL levels after surgery (Supplementa Figure 1E).

Lipids profiles

Both total cholesterol and triglycerides were unaltered during the entire follow-up, which has previously been reported to be increased, unchanged or decreased in acromegaly. Compared to the baseline, HDL, LDL and ApoB at 12 month remained unchanged, which was consistent with previous reports (Supplementa Figure 2A). Apo A1 was significantly increased at 12 months. In addition, LP(a) decreased from 280.08±193.51 mg/L at baseline to 160.42±91.03 mg/L at three months (P<0.001, Supplementa Figure 2B). Compared to the baseline, ApoB/ApoA1 significantly dropped at one year visit (0.77±0.24 vs 0.58±0.19, P=0.05, Supplementa Figure 2C).

Cardiovascular system

Compared with the baseline, daytime mean arterial pressure (MAP) was lower at one week (P=0.036), six months (P=0.009) and 12 months (P=0.013) while nocturnal MAP only declined at 12 months (P=0.014).

Echocardiography revealed significant decrease in left ventricular posterior wall at end diastole (LVPWd)
(P=0.02) at 12 months after the surgery while left ventricular internal diameter at end diastole (LVIDd), left ventricular internal diameter at end systole (LVIDs) and interventricular septal at end diastole (IVSd) only showed a declining trend at the last visit (P=0.067, P=0.15 and P=0.22) (Supplementa Figure 3, A-D). Systolic function indices such as end-diastolic volume (EDV) remained unchanged (P=0.07) at the last visit (Supplementa Figure 3F). Left ventricular fractional shortening (FS%) and ejection fraction (LVEF) remained unchanged during the whole period (Supplementa Figure 3, G-H). E/E’ ratio, the index of diastolic function, started to fall at six months (P=0.009) and got stable at 12 months (Supplementa Figure 3I). Arrhythmia was infrequent in our cohort. One patient with untreated supraventricular tachycardia recovered to normal at 6 months postoperatively (14132 beats per 24 hours at baseline vs 50 beats per 24 hours at six months).

**Pulmonary function**

Parameters for lung volumes were elevated at the baseline, indicating the existence of large lungs. The mean RV% was 134±77%, while mean RV/TLC % was 123±28% (Supplementa Figure 5, B-C). Surprisingly, lung volumes did not change over time during the whole follow-up. At the baseline, VT % was 32±139% (Supplementa Figure 5D), indicating hyperventilation status in patients with acromegaly. However, it remained unchanged even after surgical remission. At the baseline, FEV1/FVC, MEF 25% and MEF50% were elevated (105±8%, 116±39% and 114±34%, respectively) while PEF was normal (94±24%) (Supplementa Figure 5, E-H). All these indexes remained unaltered after surgery (P>0.05).

**Blood gas analysis**

Pressure of arterial carbon dioxide (Pco₂) decreased from 44.11±3.76 mmHg at baseline to 41.7±3.6mmHg (P=0.012) at six months. Buffer base (BB) decreased from 49.24±1.44 mmol/L at baseline to 48.29±1.39 mmol/L (P=0.026) at six months. Both HCO₃⁻ and base excess (BE) remain unchanged [26.47±1.71 vs 25.19±1.70 mmol/L, P=0.083; 1.4 (-0.2 to 2.4) vs 0.35 (-1 to 1.1) mmol/L, P=0.055]. Pressure of oxygen was 89.46±5.95 mmHg at the baseline while reached 94.69±6.64 mmHg at six months (P=0.052). Oxygen saturation was 96.77±0.65% at baseline, and reached 97.25±0.58% at six months (P=0.052).

**Bone metabolism**

Markers of calcium homeostasis such as 25 hydroxy-vitamin D dropped quickly at one week and continued to decrease at three months and parathyroid hormone (PTH) slightly elevated since one month visit (P=0.016) (Supplementa Figure 6, A and B). Serum phosphate fell at one month (P=0.001), but serum calcium showed no change (P=0.86). Paralleled with the changes of calcium and phosphate levels, the calcium-phosphorus ratio increased and calcium phosphorus product decreased at the one month visit (all P<0.01, Supplementa Figure 6, C and D).

**Urinary system**

Compared to the baseline, eGFR significantly decreased right after the surgery (194.01±40.30 at baseline vs 167.38±29.31 ml/min/1.73m² at 1 week, P=0.005) and persistently went down to 138.42±21.08 ml/min/1.73m² at 12 months (P<0.001). Meanwhile, serum creatinine was slightly elevated at one week and further increased at three months and 12 months. Blood urea nitrogen slightly increased since one week and remained stable at the following visits. Serum uric acid mildly increased at one month and 12 months after the surgery. However, all the above three indices remained in the normal range during the whole period. The urinary PH decreased since three months (6.09±0.33 vs 5.86±0.41, P=0.016) and remained stable (5.83±0.49) at 12 months. The total 24 hour urine volume and the urine specific gravity were unaltered till to the last visit (2465.22±865.16 vs 1886.36±815.50 ml, 1.020±0.008 vs 1.016±0.007, P=0.30 and 0.89 respectively), which indicated a normal function of posterior lobe of the pituitary.

**Liver function**
Compared with the baseline, the ALT, AST, GGT and albumin showed no change at one year visit (21.13±10.65 vs 18.75±6.17 U/L, 16.17±5.13 vs 17.00±4.02 U/L, 21.65±21.39 vs 14.17±5.84 U/L, 42.92±3.48 vs 41.25±3.75 g/L, P=0.79, P=0.37, P=0.718, and P=0.347). However, prealbumin level significantly decreased at the six months and 12 months visit (261.38±63.95 vs 217.47±49.27 vs 219.33±25.53 mg/L, P=0.025 and P=0.037), which was an important index for hepatic synthesis function.

**Complete blood count**

Compared with the baseline, the total number of white blood cells, red blood cells and platelet were unaltered at one year visit (5.56±1.78 vs 5.71±1.95*10^9/L, 4.51±0.34 vs 4.59±0.36*10^12/L, 198.58±57.61 vs 207.17±63.68*10^9/L, P=0.86, P=0.29, and P=0.67). The hemoglobin levels also remained unchanged at the last visit (136.75±13.32 vs 138.58±10.98 g/L, P=0.64).

**Blood electrolytes**

The sodium, chlorine or potassium concentrations were not changed and remained within the normal ranges during the whole follow-up. The surgery induced significant increase in magnesium level since the first week postoperatively (0.88±0.07 vs 0.94±0.08 mmol/L, P=0.004), but no further elevation was detected after then.
Supplemental References

16. Madeira M NL, de Paula Paranhos Neto F, Barbosa Lima IC, Carvalho de Mendonça LM, Gadelha MR,
Fleiuss de Farias ML. Acromegaly has a negative influence on trabecular bone, but not on cortical bone, as assessed by high-resolution peripheral quantitative computed tomography. *J Clin Endocrinol Metab.* 2013 Apr;98 1731-1741.


Supplemental Figure Legends

**Figure 1. Changes of endocrine system and glucose metabolism.** Panel A and B: TSH and FT₄ levels did not change over the whole follow-up. Panel C: FT₃ fell at one week, but returned to pre-surgical level at one month. Panel D: Testosterone levels in male patients decreased at one week and went back to normal range at three months. Panel E: All patients exhibited reduction in PRL levels since one week post-surgery. Panel F: Fasting C-peptide and 2h C-peptide levels decreased at one month and one week (P<0.01 vs. baseline for all). Panel G: AUC c-peptide of OGTT dropped since one month while there was no difference among months one, three, six, and 12. Panel H and I: IGI and INS0/BG0 significantly decreased since one week and one month. (Data are expressed as mean ± SE. *P<0.05 compared with preoperative level; #P<0.05 compared with one week level. The gray shaded area indicated the normal range. TSH: thyroid stimulating hormone; T: Testosterone; PRL: prolactin; OGTT: oral glucose tolerance test; Ins0: 0-hourinsulinof OGTT; BG0: 0-hour blood glucose of OGTT; IGI: insulinogenic index.)

**Figure 2. Changes in blood lipid profiles.** Panel A-D: Total cholesterol, HDL, LDL, triglyceride and ApoB were unaltered during the entire follow-up while ApoA1 increased at the last visit. Panel B: LP(a) decreased dramatically since three months post-operatively. Panel C: ApoB/ApoA1 significantly declined at one year visit. (Data are expressed as mean ± SE. *P<0.05 compared with preoperative level. TC: total cholesterol; TG: triglyceride; HDL: high density lipoprotein; LDL: low density lipoprotein; Apo: apolipoprotein; LP[a]: lipoprotein [a].)

**Figure 3. Changes in heart echocardiography.** Panel A-D: Echocardiography revealed significant decrease of LVPWd (P=0.028) at 12 months, while LVIDd, LVIDs and IVSd only showed a declining trend. Panel E: LVM decreased since 12 months postoperatively. Panel F-H: Systolic function indices such as EDV showed a declining trend at the last visit (P=0.05), while FS% and LVEF remained unchanged during the whole follow-up. Panel I: E/E’ ratio, index of diastolic function, started to fall significantly at six months (P=0.01). Panel J: Compared with baseline, Tei index significantly decreased at 12 months (P=0.043). (Data are expressed as mean ± SE. *P<0.05 compared with preoperative level. LVIDd: left ventricular internal diameter at end diastole; LVPWd: left ventricular posterior wall at end diastole; IVSd: left ventricular internal diameter at end systole; LVIDs: left ventricular internal diameter at end systole; LVM: left ventricular mass; EDV: end diastolic volume; FS: left ventricular fractional shortening; LVEF: left ventricular ejection fraction; Tei: total ejection isovolume index.)

**Figure 4. Changes in polysomnography.** After biochemical remission of GH and IGF-I, polysomnography revealed decrease in apnea index since one month (Panel A). Decrease in total awake time (Panel B) and awakening numbers (Panel C) and escalation in sleep efficacy (Panel D) was shown despite no statistical significance reached. Hypopnea index and total sleep time showed no change within 6 months (Panel E and F). (Data are expressed as mean ± SE. *P<0.05 compared with preoperative level. TST: total sleep time.)

**Figure 5. Changes in pulmonary function.** Panel A-D show changes in lung volume indexes. No change was observed with TLC, RV, RV/TLC and VT. Panel E-H show indexes for pulmonary ventilation function including FEV1/FVC, MEF 25, MEF50 and PEF, which were unaltered. Panel I shows DLCO% decreased at three months. Panel J-L show MIP% and MEP% were lower and P0.1/MIP% was elevated at baseline and this status was not improved during the follow-up. (*P<0.05 compared with preoperative level. Data are expressed as mean ± SE. TLC: total lung capacity; RV: residue volume; FEV1: forced expiratory volume in one second; FVC: forced vital capacity; MEF: maximal expiratory flow; PEF: peak expiratory flow; DLCO: diffusion capacity for carbon monoxide; MEP: maximum expiratory pressure; MIP: maximum inspiratory pressure. P0.1: absolute value of airway pressure drop in 100ms after airway block.)

**Figure 6. Changes of bone metabolism.** Panel A and B show vitamin D dropped quickly at one week and continued to decrease till six months and PTH slightly increased since one month visit. Panel C shows serum
phosphate fell at one month, but serum calcium was unchanged during the whole follow-up. Panel D shows that calcium phosphorus product decreased since one month visit. Panel E-H show BMD changes. A slight increase was demonstrated in mean BMD of the lumbar spine (L2-L4) and left hip (femoral neck, trochanter) since three months after surgery, but it did not reach statistical significance. (Data are expressed as mean ± SE. *P<0.05 compared with preoperative level; #P<0.05 compared with one week level. 25(OH)-vit D: 25 hydroxy-vitamin D; PTH: parathormone; Ca: calcium; P: phosphate; BMD: bone mineral density.)