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Sex differences in factors affecting spinopelvic sagittal alignment and balance: a cross-sectional observational study in a Japanese community

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Understanding how age and sex affect spinopelvic sagittal alignment is important for preventing the progression of adult spinal deformity and optimizing treatment. Here, we analyzed sex differences in age-related changes in spinopelvic sagittal alignment and balance in a Japanese community. Healthy adults (210 men and 300 women) ranging in age from their 20–80 s who participated in a general population health examination were categorized into age groups by decade according to sex. The association between changes in sagittal spinal alignment and balance, vertebral fracture, bone mineral density, bioelectrical impedance analysis, handgrip strength, and gait speed was investigated. In women, we found increased thoracic kyphosis in the 60s and pelvic retroversion in the 70s. In men, slight changes in local sagittal alignment were observed with age. Multiple linear regression analyses revealed that sagittal balance was associated with both age and vertebral fractures in men but only with age in women. Age-related changes in spinopelvic sagittal alignment differed between men and women, with men exhibiting nonstructural changes and women experiencing structural changes. In men, both age and vertebral fracture contributed to sagittal imbalance, whereas in women, only aging was associated with sagittal imbalance.

Keywords Sagittal alignment, Sagittal balance, Age-related change, Sex differences, Cross-sectional study

Adult spinal deformities (ASD) are present in 32–68% of the global population > 65 years of age and are expected to increase[1]. Patients with poor sagittal alignment tend to have poorer clinical outcomes[2]. Corrective long spinal fusion is a widely applied surgical treatment for ASD to improve disabilities. The treatment is expensive, however, and outcomes are affected by osteoporosis and other complications common in older patients[3]. Even if deformity correction is successful and clinical improvement is achieved, the indication for surgery is debatable due to its impact on daily life, such as stiffness in postoperative anterior-posterior flexion movements[4]. Spinopelvic sagittal alignment changes with age and normal values at various ages have been reported in several cohort studies[5–7]. Understanding how age and sex affect spinopelvic sagittal alignment is important for preventing ASD progression and optimizing treatment. Few studies, however, have evaluated sex differences in age-related changes in sagittal balance and the factors involved. In this cross-sectional study, we analyzed sex differences in age-related changes in spinopelvic sagittal alignment and balance.

Materials and methods

Participants

Healthy volunteers > 20 years of age who participated in the 2021 Iwaki Health Promotion Project in Hirosaki, Japan, were included in the study. The project began in 2005 and runs annual health check-ups for local residents. The health checkup, including > 2000 items per participant, aims to investigate factors related to healthy life expectancy.

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Baseline data included age, sex, height, weight, body mass index (BMI), medical history, and bone mineral density (BMD; AOS-100 scanner, Hitachi, Japan). Skeletal mass index, soft lean mass, and percent body fat (PBF) were measured by bioelectrical impedance analysis (MC-190, Tanita, Japan). Handgrip strength of each hand was measured twice, and the average of the two measurements was calculated. Gait speed was measured by timing participants as they walked 10 m at their normal pace twice, and then calculating the average time. All measurements were obtained by blinded clinical research assistants. Participants with previous spinal fusion surgery (7 cases), lumbar sacralization (7 cases), previous total hip arthroplasty (5 cases), undergoing treatment for hip osteoarthritis defined by a Kellgren-Lawrence classification 2 or higher (3 cases), and cases with missing data (4 cases) were excluded from the study.

This study was approved by the Ethics Committee of Hirosaki University Graduate School of Medicine, and all participants provided written consent before their enrollment in the study (approval number: 2022–007 H). All experiments were performed in accordance with relevant guidelines and regulations.

Assessments of spinal sagittal alignment and balance on X-ray

All participants underwent whole spine upright X-ray imaging in a neutral position with each hand placed on the ipsilateral clavicle, and the following sagittal measurements were obtained (shown in Fig. 1): Cervical lordosis (CL), Cobb angle between the inferior endplate of C2 and superior endplate of C7); thoracic kyphosis (TK), Cobb angle between the superior endplate of T4 and inferior endplate of T12); lumbar lordosis (LL), Cobb angle between superior endplate of L1 and S1); proximal lumbar lordosis (PLL), Cobb angle between superior endplate of L1 and L4; distal lumbar lordosis (DLL), Cobb angle between superior endplate of L4 and S1; lordosis distribution index (LDI), value obtained by dividing the DLL by LL; sacral slope (SS), angle between the horizontal plane and S1 superior endplate; pelvic tilt (PT), angle between the line from the center of the femoral head to the center of the sacrum and the vertical line; pelvic incidence (PI), angle between the center of the femoral head and the perpendicular line of the sacral superior endplate; T1 spinopelvic inclination (T1SPI), angle between the vertical plumbline and the line drawn from the vertebral body centroid of T1 and the centroid of the bicoxofemoral axis; T1 pelvic angle (TPA), angle between the line from femoral head center to T1 vertebral center and the line from the femoral head center to sacral center; and sagittal vertical axis (SVA), distance between the plumb line from the center of C7 vertebral body and posterior superior corner of the sacrum. The vertebrae were graded by visual inspection without direct measurements as normal (grade 0), mild deformity (grade 1, 20–25% reduction in height in the anterior, central, and/or posterior), moderate deformity (grade 2, 25–40% reduction), severe deformity (grade 3, >40% reduction)[8]. In this study, grade 2 or higher was included in the statistical analysis as vertebral fractures. All parameters were measured by orthopedic surgeons (NT and OT) using imaging software (Click measure, ONOCHI-LAB, Japan).

Statistical analysis

The data were analyzed using SPSS software (version 29.0, SPSS Inc., Chicago, IL, USA). The Mann-Whitney U and chi-square tests were used to compare baseline characteristics and sagittal parameters between men and women. Subsequent analyses were conducted separately for men and women. Tukey's Honestly Significant Difference test was used across various age groups, with sagittal parameters as the dependent variable. Linear regression analysis was performed with sagittal balance and global alignment as the dependent variable and relevant factors as independent variables. Multiple linear regression analysis was performed using the items that were $P<0.1$ in the single regression analysis as dependent variables and age and BMI as independent variables. Statistical significance was set at a level of $P<0.05$.

Results

Demographic data

Finally, 210 men and 300 women (20–85 years, mean age 52.5 ± 14.9 years) were included in the study. Baseline age, BMI, skeletal mass index, soft lean mass, BMD, and handgrip strength were significantly higher in men, while baseline PBF was higher in women. No significant differences in gait speed or vertebral fracture prevalence were detected between men and women. Among the sagittal plane parameters, SVA, CL, and LDI were significantly larger in men, while LL, PLL, PT, PI, and T1SPI were significantly larger in women. No significant differences in TPA, TK, and PLL were detected between men and women (Table 1).

Sagittal parameters of the spine

Participants were divided into 14 groups based on age decade (range 20–80 s) and sex. The study included 29 participants in their 20s (7 men, 22 women), 92 in their 30s (38 men, 54 women), 112 in their 40s (48 men, 64 women), 81 in their 50s (25 men, 56 women), 127 in their 60s (56 men, 71 women), 57 in their 70s (29 men, 28 women), and 12 in their 80s (7 men, 5 women). In men, CL was significantly greater in the 80s than in the 30s (30s vs. 80s; $P=0.035$). In women, CL was significantly greater in the 40s than in the 20s (20s vs. 40s; $P=0.037$). In men, TK, SS, and PT did not differ significantly across age groups. In women, TK was greater in the 60s than in the 40s (60s vs. 40s; $P=0.033$), PT was greater in the 70s than in the 50s (70s vs. 50s; $P=0.017$), while SS did not differ significantly across age groups (Fig. 2). No significant age-related changes in LL, PLL, DLL, or PI were observed in either men or women. Although no statistically significant differences were detected, a gradual increase in PLL and decrease in DLL were observed after middle age (Fig. 3). In men, SVA was greater in the 80s than in the 50s (50s vs. 80s; $P=0.023$), while the TPA did not differ significantly across age groups. In women, SVA was significantly greater in the 60s than in the 40s (40s vs. 60s; $P=0.012$), and TPA was significantly greater in the 70s than in the 50s (50s vs. 70s; $P=0.011$) (Fig. 4).

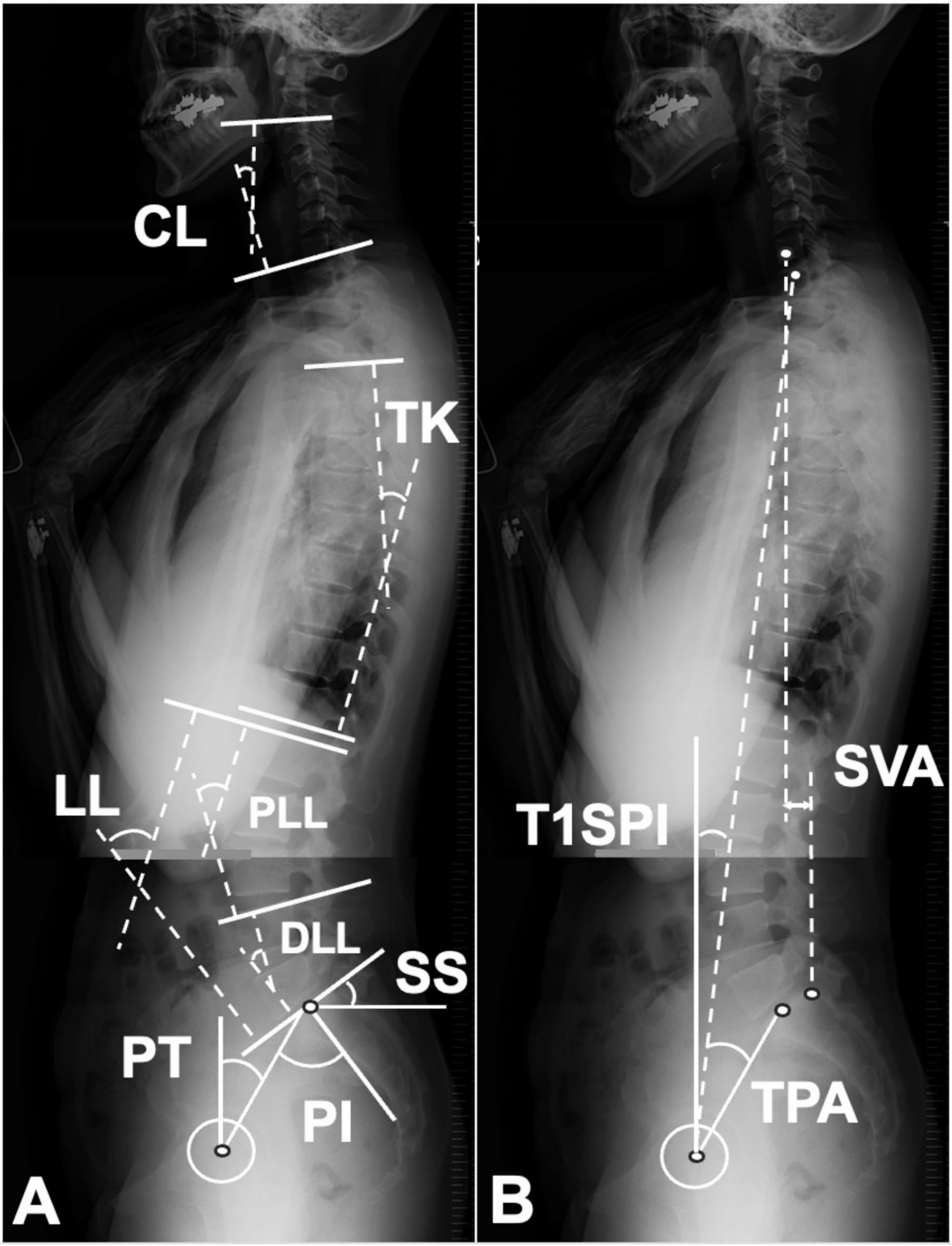

Fig. 1. Assessment by standing X-ray sagittal image. Assessments of spinopelvic sagittal alignment (**A**), sagittal balance and global alignment (**B**) are shown. CL, cervical lordosis; TK, thoracic kyphosis; LL, lumbar lordosis; PLL, proximal lumbar lordosis; DLL, distal lumbar lordosis; SS, sacral slope; PT, pelvic tilt; PI, pelvic incidence; SVA, sagittal vertical axis; TPA, T1 pelvic angle; T1SPI, T1 spinopelvic inclination.

Factors related to sagittal balance

Factors associated with an increased SVA are presented in Table 2. In men, univariate linear regression identified age and vertebral fractures as associated factors, while multivariate analysis confirmed that age (B = 0.312, 95% CI: 0.048–0.576, P = 0.021) and vertebral fractures (B = 13.102, 95%CI: 1.503–24.701, P = 0.027) remained

Characteristics	Men (N=210)	Women (N=300)	P value
Age	54.1±15.0	51.4±14.8	**0.045**
BMI (kg/㎡)	23.9±2.9	22.1±3.4	**<0.001**
SMI (kg/㎡)	7.7±0.7	6.0±0.6	**<0.001**
SLM (kg)	50.7±6.0	35.8±3.8	**<0.001**
PBF (%)	22.0±5.8	29.5±6.8	**<0.001**
BMD T score (%)	100.1±11.1	94.9±16.8	**0.004**
Handgrip strength (kg)	38.8±6.9	24.1±3.9	**<0.001**
Gait speed (m/s)	1.4±0.2	1.5±0.2	0.606
Presence of VFs	28	29	0.196
Sagittal parameters			
SVA (mm)	7.7±27.3	-5.3±25.1	**<0.001**
TPA (°)	11.5±6.5	11.6±7.7	0.907
CL (°)	10.4±11.1	5.2±12.9	**<0.001**
TK (°)	31.8±10.4	30.7±12.0	0.156
LL (°)	45.6±11.8	49.6±12.2	**<0.001**
PLL (°)	15.0±8.3	20.0±9.1	**<0.001**
DLL (°)	30.5±8.1	29.7±9.3	0.337
LDI	71.1±35.8	60.6±18.2	**<0.001**
SS (°)	33.3±9.1	35.7±9.1	**0.004**
PT (°)	15.9±6.7	17.4±7.8	**0.023**
PI (°)	49.1±10.0	53.1±11.6	**<0.001**
T1SPI (°)	4.4±2.5	5.8±2.2	**<0.001**

Table 1. Baseline characteristics and sagittal parametersResults are shown as mean ± standard deviation of the number of participants. BMI, body mass index; SMI, skeletal muscle mass index; SLM, soft lean mass; PBF, percent body fat; BMD, bone mineral density; VFs, vertebral fractures; SVA, sagittal vertical axis; TPA, T1 pelvic angle; CL, cervical lordosis; TK, thoracic kyphosis; LL, lumbar lordosis; PLL, proximal lumbar lordosis; DLL, distal lumbar lordosis; LDI, lumbar distribution index; SS, sacral slope; PT, pelvic tilt; PI, pelvic incidence; T1SPI, T1 spinopelvic inclination.

significant related factors. In women, univariate linear regression analysis identified age and BMD, while multivariate analysis confirmed that age (B = 0.544, 95% CI: 0.292–0.796, $P < 0.001$) remained a significant related factor. Factors associated with an increased TPA are presented in Table 3. In men, univariate linear regression identified age, BMI, PBF, and vertebral fractures as associated factors, and multivariate analysis confirmed that age (B = 0.085, 95% CI: 0.02–0.151, $P = 0.011$) remained a significant related factor. In women, univariate linear regression analysis identified age, BMI, BMD, and PBF as associated factors, and multivariate analysis confirmed that age (B = 0.124, 95% CI: 0.057–0.192, $P < 0.001$) remained a significant related factor.

Discussion

Our findings revealed sex differences in spinopelvic sagittal alignment and balance. In men, the sagittal balance of the spine shifted forward with age, with local alignment changing only slightly. In women, the sagittal balance shifted anteriorly due to structural changes, including age-related thoracic kyphosis and compensatory pelvic retroversion. Vertebral fractures were associated with deteriorating sagittal balance only in men, likely due to a lower compensatory capacity compared with women.

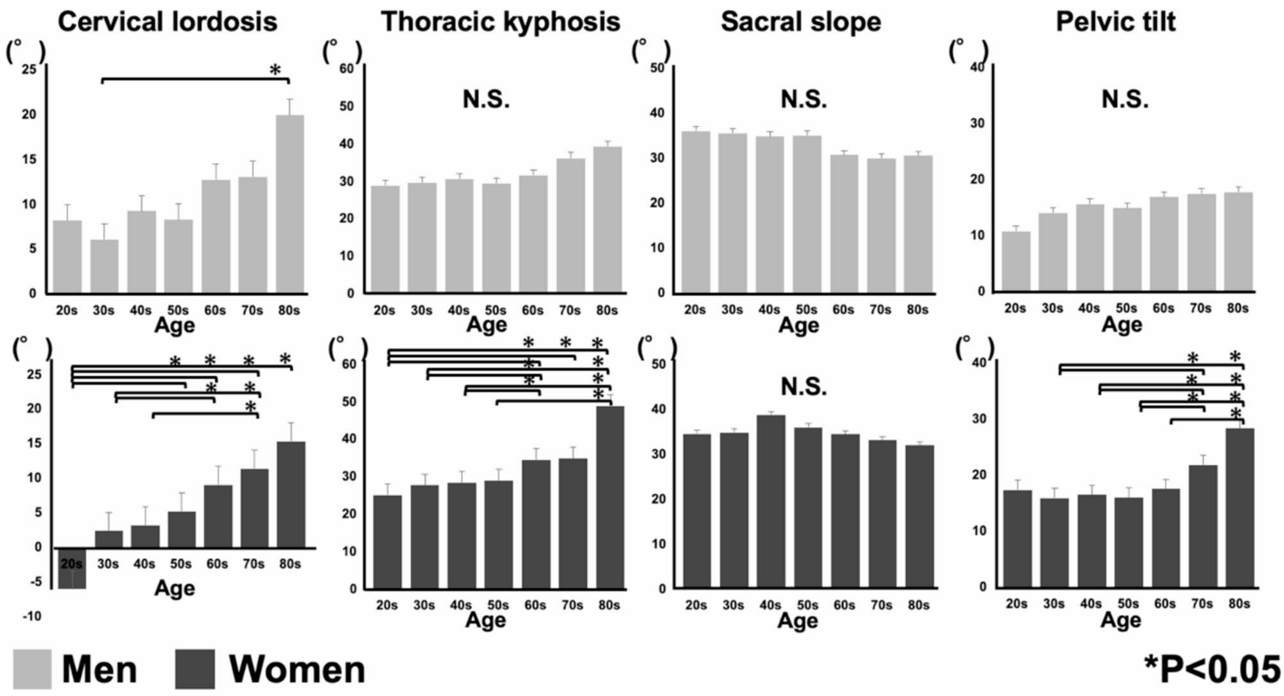

Fig. 2. Age-related changes in spinal sagittal alignment. In men, cervical lordosis (CL) increased in the 80s, but there were no significant changes in thoracic kyphosis (TK), sacral slope (SS), or pelvic tilt (PT) across age groups. In women, CL increased in the 50s, TK in the 60s, and PT in the 70s. There were no significant changes in SS. NS, not significant.

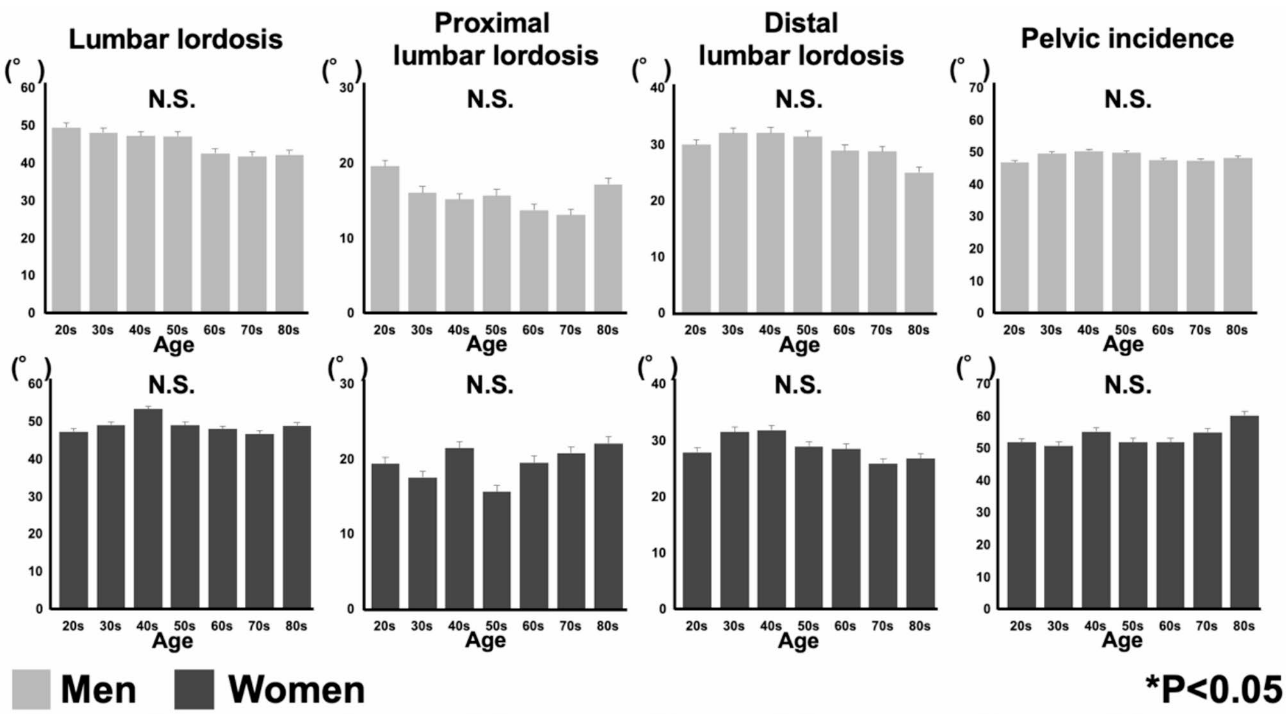

Fig. 3. Age-related changes in lumbar spine and pelvic incidence. No significant age-related changes in lumbar lordosis (LL), proximal lumbar lordosis (PLL), distal lumbar lordosis (DLL), or pelvic incidence (PI) were detected in either sex between age groups. A gradual PLL increase and DLL decrease were observed after middle age. NS, not significant.

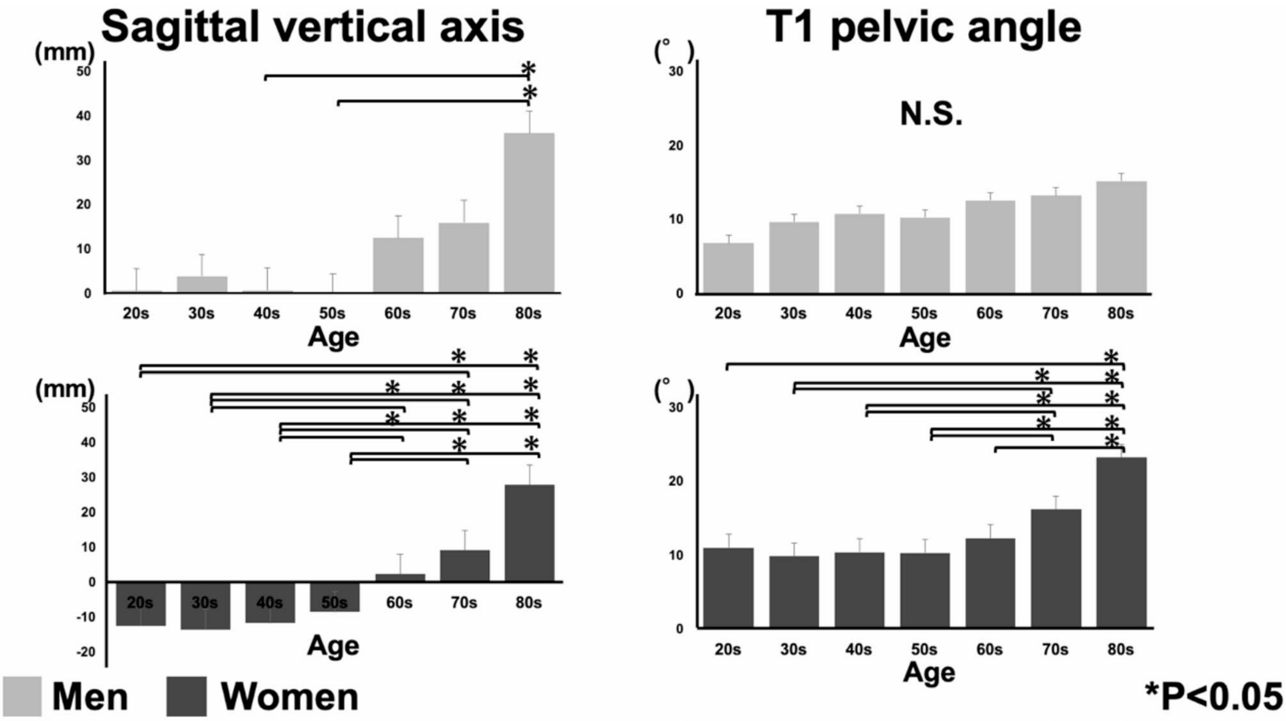

Fig. 4. Age-related changes in sagittal balance and global alignment. In males, the sagittal vertical axis (SVA) showed a significant increase only in the 80s, while the T1 pelvic angle (TPA) showed no significant change between the various age groups. In women, the SVA was negative from young to middle age, with a significant increase after age 60; TPA increased significantly after age 70. NS, not significant.

Several recent studies investigated the sagittal alignment of the spine and pelvis. In a cross-sectional study, Uehara et al. found that age-related changes in sagittal alignment begin earlier in women than in men[5]. Oe et al. reported that age-related changes in all age groups are greater in women than in men based on a 4-year longitudinal study[6]. Earlier reports focused on changes in local spinal alignment. Takemitsu et al. reported a decrease in lumbar lordosis with age in patients with low back pain[9], and Kim et al. reported an increase in thoracic kyphosis with age in a comparison of young and older volunteers[10]. These two last reports were cohort studies comparing alignment between different age groups, but no definite conclusions were reached.

Sagittal alignment differs between men and women. In a study of healthy young men and women, Hesarikia et al. reported greater lumbar lordosis in women but found no differences in other parameters or sagittal balance[11]. The participants in their study were quite young, with an average age of 13, making it unlikely to reflect the changes that occur in women during puberty and childbirth. Lumbar-pelvic alignment temporarily increases in women in their 40s[7], a trend we also observed in the present study. Janssen et al., comparing 3D-constructed X-rays of young adult men and women, found that women have a greater posterior tilt of the thoracic spine relative to the lumbar spine compared to men[12]. In this study, the T1SPI, which is the angle of inclination of the thoracic spine and pelvis, was significantly larger in women than in men, and the results are consistent with findings from previous studies. This may be the result of the physiologic posture of women, who have a greater posterior inclination of the pelvis than men, helping to maintain global alignment.

In this study, women showed an increase in thoracic kyphosis from their 60s onwards and an increase in the posterior pelvic tilt from their 70s onwards. No significant difference in lumbar lordosis, however, was detected between the age groups. These findings suggest that in women, the increase in thoracic kyphosis due to aging may be compensated for by the backward tilt of the pelvis to maintain sagittal balance. On the other hand, in men, although the increase in thoracic kyphosis, lumbar lordosis, and posterior pelvic tilt did not differ significantly between age groups, a gradual increase was observed. These findings strongly suggest that in women, the pelvis compensates for age-related spinal deformities, whereas in men, such compensatory changes are minimal. This may be due to the lower mobility of the spine-pelvis in men compared to women, resulting in a reduced need for compensation as age-related changes are less pronounced. Mizukoshi et al. compared computed tomography scans of participants in standing and supine positions and reported a greater range of pelvic motion in women[13]. Our findings, which show that compensation for spinal deformity through posterior pelvic tilt occurs primarily in women, align with their results. The age-related changes in sagittal balance and global alignment observed in this study are shown schematically in Fig. 5. In men, changes observed in the local alignment of the spine and pelvis were gradual, and the entire spine and pelvis shifted forward, with sagittal balance shifting forward while maintaining global alignment. In women, on the other hand, sagittal balance shifted forward in line with the increase in global alignment due to increased kyphosis of the thoracic spine and posterior pelvic tilt. This may be due to differences in the mechanisms underlying age-related changes between men and women.

Men	Crude		Adjusted	
	B (95%CI)	P value	B (95%CI)	P value
Age	0.43 (0.188-0.672)	**<0.001**	0.312 (0.048-0.576)	**0.021**
BMI	0.532	0.41	-	0.304
SMI	-1.185	0.68	-	-
SLM	-0.196	0.534	-	-
PBF	0.516	0.11	-	-
BMD	5.368	0.809	-	-
Handgrip strength	-0.331	0.226	-	-
Gait speed	-15.438	0.122	-	-
Presense of VFs	18.693 (8.037-29.349)	**<0.001**	13.102 (1.503-24.701)	**0.027**

Women	Crude		Adjusted	
	B (95%CI)	P value	B (95%CI)	P value
Age	0.567 (0.384-0.749)	**<0.001**	0.544 (0.292-0.796)	**<0.001**
BMI	0.386	0.366	-	0.818
SMI	-1.195	0.62	-	-
SLM	-0.41	0.283	-	-
PBF	0.186	0.381	-	-
BMD	-57.068 (-82.63 - -31.505)	**<0.001**	-	0.632
Handgrip strength	-0.415	0.267	-	-
Gait speed	-0.419	0.957	-	-
Presense of VFs	7.338 (-2.273-16.95)	0.134	-	-
				Linear regression analysis, Dependent variable: SVA

Table 2. Linear regression analysis with sagittal vertical axis as the dependent variable. BMI, body mass index; SMI, skeletal muscle mass index; SLM, soft lean mass; PBF, percent body fat; BMD, bone mineral density; VFs, vertebral fractures.

In this study, sagittal balance increased with age in both men and women but did not significantly associate with body composition, grip strength, or walking speed in men or women. Hori et al.[14] reported a negative correlation between trunk muscle mass and the sagittal vertical axis based on bioelectrical impedance analysis in a multicenter cross-sectional study. A biologic and pathologic age-related decrease in skeletal muscle mass and strength is commonly referred to as sarcopenia, and an age-related reduction in the cross-sectional area and fatty degeneration of the paraspinal muscles is reported[15]. Aging decreases body flexion-extension torque and increases trunk sarcopenia based on a survey of the 2013 Iwaki Health Promotion Project[16]. In this study, grip strength and walking speed, which positively correlate with back muscle strength as factors related to sarcopenia, were also included as alternative indicators, but no significant correlation was found. In the linear regression analysis, age was significantly associated with the deterioration of sagittal balance and global alignment in both men and women, but vertebral fracture was significantly associated with sagittal imbalance only in men. Hu et al. reported that patients with osteoporosis who have vertebral fractures have greater global alignment than healthy people[17]. These findings suggest that the age-related changes in the spine and pelvis in men are slight between each age group, and that the effect of local alignment changes due to vertebral fractures on sagittal balance may be relatively greater than in women.

Men	Crude		Adjusted	
	B (95%CI)	P value	B (95%CI)	P value
Age	0.104 (0.047-0.161)	**<0.001**	0.085 (0.02-0.151)	**0.011**
BMI	0.253 (-0.045-0.552)	**0.096**	-	0.236
SMI	-0.039	0.954	-	-
SLM	-0.082	0.273	-	-
PBF	0.158 (0.08-0.307)	**0.038**	-	0.979
BMD	-2.211	0.673	-	-
Handgrip strength	-0.043	0.508	-	-
Gait speed	-1.111	0.638	-	-
Presense of VFs	3.706 (01.167-6.245)	**0.004**	-	0.117

Women	Crude		Adjusted	
	B (95%CI)	P value	B (95%CI)	P value
Age	0.121 (0.063-0.178)	**<0.001**	0.124 (0.057-0.192)	**<0.001**
BMI	0.37 (0.117-0.624)	**0.004**	-	0.508
SMI	1.016	0.168	-	-
SLM	0.071	0.543	-	-
PBF	0.125 (-0.001-0.252)	**0.053**	-	0.729
BMD	-14.472 (-22.369 - -6.575)	**<0.001**	-	0.226
Handgrip strength	-0.129	0.259	-	-
Gait speed	-0.66	0.78	-	-
Presense of VFs	1.51	0.314	-	-
Linear regression analysis, Dependent variable: TPA				

Table 3. Linear regression analysis with T1 pelvic angle as the dependent variable. BMI, body mass index; SMI, skeletal muscle mass index; SLM, soft lean mass; PBF, percent body fat; BMD, bone mineral density; VFs, vertebral fractures.

Been et al. referred to the interaction between spinal-pelvic alignment and spinal disorders, and emphasized the importance of identifying patients at risk of spinal disorders at an early stage[18]. The PI is greater in women than in men, which is associated with a greater compensatory function for sagittal imbalance[19]. In addition, recent research on lumbar alignment demonstrated that a larger PI is associated with the onset and progression of lumbar degenerative spondylolisthesis[20]. Patients with this condition exhibit a decrease in DLL and a relative increase in PLL, highlighting pathologic interactions with sagittal alignment as a key area of focus[21]. In the present study, while women had significantly larger LLs than men, we found no difference in DLLs between sexes, and as a result, men had a significantly larger LDI. In addition, a gradual increase in PLL and a decrease in DLL were observed in older age groups, suggesting degeneration of the lower lumbar spine and compensatory changes in response. These structural differences may support the idea that compensatory changes are more likely in women and that sex differences influence the risk of developing spinal diseases, such as degenerative spondylolisthesis.

Sagittal imbalance is a degenerative spinal change observed in ASD, and a cascade from sagittal balance, compensated sagittal balance, and decompensated sagittal imbalance has been proposed[22]. More recently, a fourth stage, characterized by sagittal imbalance with failure of pelvic compensation, was proposed[23]. Han et al. reported that degeneration of the paraspinal muscles is a risk factor for progression from compensated sagittal

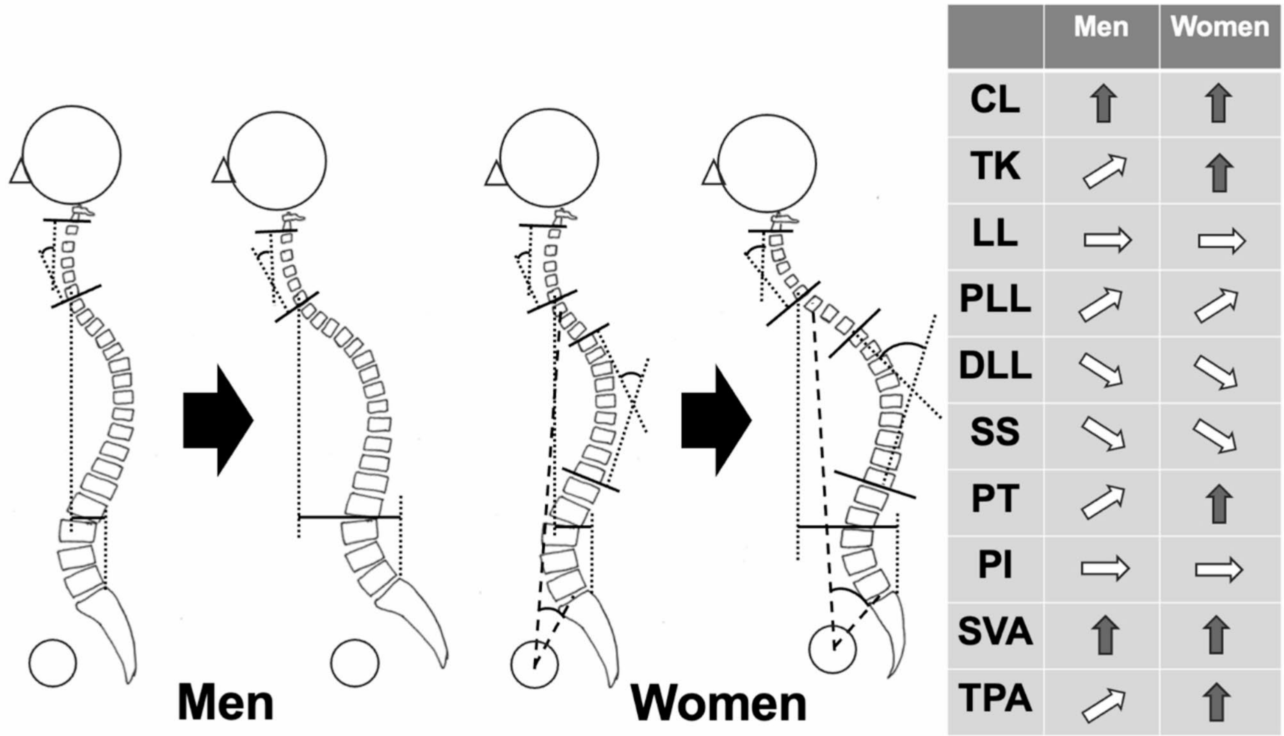

Fig. 5. Sex differences in age-related changes in spinopelvic sagittal alignment. In men, the sagittal balance increased with little local alignment change. In women, the sagittal balance shifted forward, accompanied by a structural change in sagittal alignment. Abbreviations: CL, cervical lordosis; TK, thoracic kyphosis; LL, lumbar lordosis; PLL, proximal lumbar lordosis; DLL, distal lumbar lordosis; SS, sacral slope; PT, pelvic tilt; PI, pelvic incidence; SVA, sagittal vertical axis; TPA, T1 pelvic angle.

balance to decompensated sagittal imbalance, in addition to being an initiator of posterior pelvic tilt in patients with lumbar spinal stenosis[24]. According to our research, these factors were not significantly associated with static sagittal balance or global balance, and aging, vertebral fracture, and compensatory changes may be the main causes of age-related changes. On the other hand, dynamic sagittal imbalance worsens after 10 min of walking, even if it is maintained in a static posture, and hidden dynamic sagittal imbalance, even in healthy subjects, cannot be ruled out[25]. Further research is essential, as compensatory deficits due to degenerative degeneration and reduced endurance due to paraspinal muscle degeneration may influence these mechanisms.

Limitations.

This study has several limitations. First, as it was a cross-sectional study, it was not possible to identify the causal relationship between various factors, and a longitudinal study is needed. Second, the participants in this study were limited to healthy adult volunteers able to walk independently, and the number of participants with severe adult spinal deformities was small. Specifically, 20 participants (4%) had a sagittal vertical axis of more than 50 mm, which is considered an indicator of sagittal imbalance. In addition, the proportion of young and older participants was low, with the largest number of participants being middle-aged. This may have affected the variability of the mean values and statistical accuracy. Third, orthopedic examination and imaging evaluation were limited in this study. Because frontal and lower extremity radiographs were not performed, the study may not have taken into account any compensatory changes or asymmetries in the coronal plane. Despite these limitations, this large-scale cohort study revealed age-related changes in the sagittal plane of the spine and pelvis that differ by sex. Future studies based on these data may facilitate the investigation of the mechanisms and treatment strategies for adult spinal deformities.

Conclusion
Our findings indicated that age-related changes in the sagittal alignment of the spine and pelvis were localized in men and global in women. Sagittal balance increased non-structurally in men and structurally in women with age. In men, age and vertebral fracture were associated factors, while in women, only age was an associated factor.

Data availability
Data cannot be shared publicly because of the ethical concerns. Data are available from the Hirosaki University COI Institutional Data Access / Ethics Committee (contact via e-mail: coi@hirosaki-u.ac.jp) for researchers who meet the criteria for access to the data. Researchers need to be approved by research ethics review board at the organization of their affiliation.

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Author contributions

N.T. and W.K. wrote the programs, designed the analysis. N.T., O.T., and W.K. analyzed the data, and wrote the paper. K.G., N.Y., A.K., W.K, and I.Y. supervised the conception and edited the manuscript. All authors discussed and commented on the manuscript.

Declarations

Competing interests

The authors declare no competing interests.

Ethics approval

The study was approved by the Ethics Committee of Hirosaki University Graduate School of Medicine (2008-

025 and 2018-063) and conducted in accordance with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. All participants provided written informed consent before participation.
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Additional information

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