**Independent Relationships Between Bone Mineral Density, Regional Body Fat and Insulin Sensitivity in White Males**

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**Abstract :**

*Background.* Adiposity and insulin sensitivity may affect bone mineral density (BMD) but the confounding effect of weight hinders discrimination of independent associations. We explored whether regional fat masses and insulin sensitivity are independently related to BMD.

*Materials and Methods.* Relationships between total and regional body fat, insulin sensitivity and measures of BMD in 8 different regions were evaluated in a cross-section of 590 generally healthy, white males, 274 of whom received measurement of insulin sensitivity (Si) using the intravenous glucose tolerance test. Measurements included total, android and gynoid fat and lean body mass and regional BMDs by dual-energy X-ray absorptiometry. Linear regression analyses were combined in a mediation analysis to explore associations with each regional BMD.

*Results.* Weight correlated positively with total fat mass (R2=0.67, p<0.001) and negatively with Si (R2=0.14, p<0.001). Body composition measures were consistently positively related to BMD in all regions except lumbar and thoracic spine. Accounting for body weight rendered negative the majority of associations between total and regional fat masses and BMDs. An independent association between android fat and spine BMD was particularly apparent. Si was positively associated with total and limb BMD (p<0.01) specifically among exercisers. Accounting for Si diminished the associations of total fat (negative) and lean body mass (positive) with total and limb BMD.

*Conclusion.* Android fat is independently negatively associated with spine BMD. Among those taking exercise, increased insulin sensitivity is associated with higher limb BMD and may underlie positive associations between lean body mass and BMD.

Keywords: android fat, insulin sensitivity, dual energy X-ray absorptiometry, bone mineral density, mediation analysis

**Introduction**

 Variation in bone mineral density (BMD) has been related to variation in weight ([1](#_ENREF_1)), adiposity ([2](#_ENREF_2)), insulin sensitivity and plasma insulin concentrations ([3-5](#_ENREF_3)). However, on account of mutual confounding and the co-linearity between these explanatory measures, there is uncertainty regarding the independent contribution each makes to variation in BMD ([6](#_ENREF_6)). For example, the mechanical loading effect of increasing weight is associated with increasing BMD ([1](#_ENREF_1)) but increasing weight is often associated with increasing fat mass. Therefore, any negative association of increasing fat mass with BMD could be masked by the positive effect of increasing weight. Moreover, with increasing fat mass there is decreasing insulin sensitivity, so any negative association of decreasing insulin sensitivity with BMD could be masked by the positive effect of the increased weight associated with increasing fat mass. Further potential confounding comes from the positive association of insulin sensitivity with increased lean body mass ([3](#_ENREF_3)) and, although insulin resistance may diminish any effect of insulin on bone metabolism, this could be countered by the hyperinsulinaemia that accompanies insulin resistance.

 Some of the consequences of such mutual confounding may be seen in findings from recent studies. For example, Zhao et al found that fat mass was positively correlated with bone mass, but became negatively associated with bone mass when weight was adjusted for ([7](#_ENREF_7)). Similarly, Lawlor et al showed that the direction of association between increasing fasting insulin concentration (an index of decreasing insulin sensitivity) and BMD changed from positive to negative when total fat mass was adjusted for ([4](#_ENREF_4)).

 A further complication is that body fat is not homogenous; different fat depots may differ in their metabolic characteristics and may consequently differ in their effects on BMD. For example, recent meta-analyses found that obesity ([2](#_ENREF_2)) was associated with reduced risk of fractures but abdominal obesity was associated with *increased* risk, specifically in the hip region ([8](#_ENREF_8)).

 To identify independent associations between fat and lean tissue, weight and insulin sensitivity and BMD, discriminatory measures of regional body composition and insulin sensitivity are needed. Moreover, rather than considering inter-relationships between these variables in terms of mutual confounding, greater clarity may be achieved by considering adiposity as a primary influence on BMD, with weight and insulin sensitivity (and other correlates of adiposity and BMD) as possible mediators of the effect of adiposity. In the present study, we have explored associations between dual-energy X-ray absorptiometry (DXA) measures of regional body fat and lean body masses with BMD in 8 regions. We have then combined body composition measures with weight and then with weight plus intravenous glucose tolerance test (IVGTT) measures of insulin sensitivity to identify independent contributions of body composition, weight and insulin sensitivity to variation in BMD in different body regions.

**Material and Methods**

*Participants:*

 The Heart Disease and Diabetes Risk Indicators in a Screened Cohort (HDDRISC) study is a prospective study of 1192 white males recruited as part of a company health screening program in United Kingdom, with data acquisition from 1971 to 2000. Participants were senior executives, apparently healthy and working or recently retired, who were taking few or no medications. Measurements were carried out by a historically continuous research team at a dedicated metabolic day ward. Each participant provided a full medical history including details of smoking, alcohol consumption, exercise and medication. Physical examination included height and weight assessment. As well as lipids and lipoproteins, haematology, liver function tests and electrolytes, blood laboratory measurements included fasting plasma glucose (FPG) and fasting plasma insulin (FPI) concentrations. A second sample for FPG and FPI was taken 5 minutes later, for calculation of mean values. Quality control of laboratory measurements was monitored throughout the study. Further details have been described previously ([9](#_ENREF_9)). The study received ethics committee approval and participants gave their written informed consent. Participants were excluded from the present analysis if they had fasting plasma glucose ≥7mmol/L, a history of diabetes or were taking glucose lowering medications.

*Dual-energy X-ray absorptiometry:*

DXA scanning was offered to HDDRISC participants between 1989 and 1998. Scans were performed using a Lunar Radiation Corporation (Madison, WI, USA) scanner to measure regional BMD and body composition ([10](#_ENREF_10)). Regional BMD measurements were recorded for total (whole body), limb (with separate measurements for leg - including femoral neck - and arm), pelvis, spine (with separate measurements for lumbar and thoracic regions), rib and head. Body composition measures included total, android and gynoid fat masses and total lean body mass. To distinguish regional measures, proprietary DXA scan software was used as previously described ([11](#_ENREF_11)). The android fat region was represented by an upper horizontal border underneath the chin with vertical borders passing down the ribs and a lower border formed by diagonal lines passing through the hip joints and meeting at the perineum; the gynoid fat region was represented by the tissue below these diagonal lines ([11](#_ENREF_11)). DXA measurement precision estimates for total body measures were 0.65% for BMD, 2.73% for fat mass and 1.47% for lean mass ([10](#_ENREF_10)). Long term precision and accuracy were monitored by daily scanning of the Lunar mineral equivalent phantom.

*Insulin sensitivity and insulin resistance:*

In those consenting to undergo the procedure, insulin sensitivity, Si, was measured by minimal model analysis ([12](#_ENREF_12)) of IVGTT plasma glucose and insulin concentrations, as previously described ([13](#_ENREF_13), [14](#_ENREF_14)). Also, the homeostasis model assessment index of insulin resistance (HOMA-IR) was calculated from mean FPG and FPI concentrations as an index of insulin resistance ([15](#_ENREF_15)). A high HOMA-IR signifies low insulin sensitivity.

*Data and statistical analysis:*

Smoking was coded as 0, <15 (light) or ≥15 (heavy) cigarettes per day, alcohol consumption as 0, <28units per week (light) or ≥28 units per week (heavy) and exercise habits as none, moderate and regular (≥3 periods of ≥20min of exercise to breathlessness per week). Data was analysed using STATA 13 for Windows (Stata, College Station, TX, USA). Individual characteristics were summarised as median and interquartile range (IQR) for continuous variables and percentages and number of observations (n) for categorical variables. Body composition measures were expressed as both mass and as percentage of body weight. However, increasing fat mass was largely responsible for increasing weight (71% of the variance in weight explained). This made of fat mass percentage a proxy measure for fat mass (36% of the variance in weight explained). As a consequence, lean mass percentage decreased with increasing weight (R=-0.62). Because of these confounding associations, mass measures were used in subsequent analyses, rather than mass percentages. Independent associations were investigated using multiple linear regression analysis with continuous variables standardised according to numbers of standard deviations from the mean. Multivariable analyses were structured according to a mediation analysis, whereby the impact of sequentially introducing additional explanatory variables on the regression coefficients for the associations between regional fat masses and BMDs can suggest factors that might be responsible, mechanistically, for these associations ([16](#_ENREF_16)). This analysis of modifications to associations remains cross-sectional, however, so causation cannot be inferred. Three basic models of variation in each of the 8 BMD regions were explored. In Model 1, total, android or gynoid fat or lean body mass was entered as a predictor variable along with age, height, smoking, alcohol intake and exercise habit. In Model 2, the same predictors were included plus weight. In Model 3, again the same predictors were included plus weight and insulin sensitivity, Si, or insulin resistance, HOMA-IR. Regression coefficients were compared by two-tailed z-test. Interactions between Si and exercise in influencing associations between regional body composition measures and BMDs were also explored. Associations between regional body composition and BMDs and differences between regression coefficients significant at p <0.05 were considered for interpretation. Choice of variables in each test of association undertaken was weighted by existing evidence, thus rendering the universal null hypothesis and a correction for multiple testing inapplicable ([17](#_ENREF_17)). However, evidence relating to each of the 8 measures of regional BMD varies in strength and, although total fat has been investigated, a distinction between android and gynoid fat has rarely been made. Therefore, in drawing conclusions from our full model (Model 3) regarding the 8 BMD measures and the 2 regional fat measures, weight is give to significances less than 0.05 divided by 8 x 2, i.e. 0.003. All multivariable models were checked for collinearity using the variation inflation factor, which did not exceed 3.3 in the models we describe. ([18](#_ENREF_18)).

**Results**

Of the 590 participants in the HDDRISC study who met inclusion criteria for the current analysis and underwent a DXA scan, 274 consented to undergo an IVGTT. Table 1 summarizes characteristics for all participants. The median (range) age and BMI were 50.4 (43.7,58.6) years and 25.7 (24.1,27.8) kg/m2, respectively. Exercise habit was reported as 47% sedentary; 32% moderate and 21% regular. Prescription drug use was relatively low: 2% were taking lipid-lowering agents; 6% anti-hypertensives and 1% uric acid-lowering agents. One participant had been prescribed prednisolone for asthma; otherwise steroid use was restricted to 4 participants using steroid creams and 14 using asthma inhalers. No participant was being treated for osteoporosis.

*Weight, body composition, Si and individual characteristics*

Weight was positively correlated with total fat mass (R2=0.67, p<0.001) and negatively with Si (R2=0.14, p<0.001). Total fat mass was negatively correlated with Si (R2=0.19, p<0.001). In univariable and multivariable regression, weight was positively associated with all regional body composition measures (Supplementary Table 1). Si was negatively associated with each body composition measure in univariable analysis but, in multivariable analysis, Si became positively associated with lean body mass. In univariable analysis, age was negatively associated with each body composition measure and heavy smoking was positively and exercise negatively associated with fat mass measures.

*Individual characteristics and BMD*

In brief (results not shown), in both univariable and multivariable analyses, age was independently, positively associated with spine BMD, height with rib BMD and moderate exercise with total, leg, arm, pelvis and lumbar spine BMD.

*Regional body fat masses and BMD*

In Model 1, with neither weight nor Si included, total, android and gynoid fat masses were positively associated with most BMDs (Tables 2a-2c). However, total and android fat were negatively associated with lumbar spine BMD and not associated with thoracic spine BMD. In Model 2, with inclusion of weight, all positive associations between regional fat masses and BMDs were eliminated, indicating that the positive associations were an effect of the positive contribution of fat mass to body weight. In Model 2, moreover, negative associations emerged between total and android fat and thoracic spine, rib and head BMDs and these associations were sustained in Model 3 with further inclusion of Si. Total fat mass was also negatively associated with total and leg BMDs in Model 2 but with inclusion of Si in Model 3 these associations were eliminated, suggesting possible mediation of this association by the low insulin sensitivity associated with increased total fat mass. With inclusion of weight in Model 2, regression coefficients differed significantly from those in Model 1 for associations between total and android fat mass and all BMDs. Regression coefficients also differed significantly between Models 1 and 2 for associations between gynoid fat mass and total, leg, arm and rib BMDs. However, despite the elimination of negative associations between total fat mass and total and leg BMDs by inclusion of Si, regression coefficients did not differ significantly between Models 2 and 3.. Positive associations between android and gynoid fat masses and pelvis BMD were eliminated when variation in weight was taken into account in Model 2, suggesting weight was the primary factor in these associations with pelvis BMD.

*Lean body mass and BMD*

Lean body mass was positively associated with all measures of regional BMD in Model 1 and most BMDs in Model 2 with weight taken into account (Table 2d). Regression coefficients for lean body mass and BMD differed significantly between Models 1 and 2 with inclusion of weight only for the arm and rib BMD analyses and then only weakly (p<0.05). Inclusion of Si in Model 3 weakened or eliminated the positive associations between lean body mass and total, leg, arm and pelvis BMD suggesting that the positive associations of lean body mass with these BMDs might be partly mediated by the increased Si associated with increased lean body mass. Nevertheless, regression coefficients did not differ significantly between Models 2 and 3.

*Weight, insulin sensitivity and BMD*

In multivariable analysis, without including any body composition measure as an explanatory variable, weight was positively associated with all BMD measures except lumbar spine (Table 2a), but with inclusion of total and android fat masses, in Models 2 and 3, weight was significantly, positively associated with BMD in all regions, including lumbar spine (Tables 2a and b). With inclusion of lean body mass, weight was associated with all BMD measures except thoracic spine BMD. (Table 2 d). Si was positively associated with total, arm and leg BMD, independently of all body composition measures. However, sub-analyses in non-exercisers or exercisers revealed that the positive association between Si and BMD was not apparent in non-exercisers (n=120, coefficient -0.004 (95% CI -0.190,0.182), p=0.9) and it was only among exercisers that the significant association was seen (n=152, coefficient 0.276 (95% CI 0.123,0.429), p<0.001). In this model, no difference between regular exercise and moderate exercise was apparent (coefficient -0.269 (95%CI -0.583,0.044), p=0.09). The fasting measure of insulin resistance, HOMA-IR, behaved very similarly to the measure of insulin sensitivity, Si, albeit inversely (not shown).

*The estimated effect of obesity on BMD:* The independent impacts of android fat mass, insulin sensitivity and weight on regional BMDs in obese people relative to those of normal weight were estimated using the coefficients from multivariable Model 3. The mean BMI among normal weight (BMI<25kg/m2) participants in the HDDRISC study was 23.3kg/m2 and among the obese (BMI ≥30kg/m2) was 32.3kg/m2, a 9kg/m2 difference. Based on the regression coeffcient for BMI as a predictor of Si in HDDRISC participants, the expected effect of a 9kg/m2 increase in BMI on Si would be a reduction of 2.42 /min/mU/L. Using the Model 3 coefficients, the independent effects of an increase in android fat mass or weight of 9kg on regional BMD and the effects of a 2.42 /min/mU/L decrease in Si were calculated (Figure 1). The negative effect of obesity, mediated by a reduction in Si on limb BMD, was balanced by the positive effect of increased weight (e.g. arm BMD: -3.3% vs +3.6%). However, the negative effect of obesity mediated by android fat on spine BMD outweighed the positive effects of increased weight (e.g lumbar spine BMD: -22.1% vs +9.1%).

**Discussion**

 Original findings in our analysis were that android fat mass was negatively related to spine (lumbar and thoracic) BMD and insulin sensitivity was positively related to limb (leg and arm) BMD. The ubiquitous positive effect of weight on BMD was also apparent, extending even to head BMD, suggesting a systemic as well a mechanical effect of weight on BMD. Based on the magnitude of the independent effects we observed, we estimated that the negative effect on limb BMD of the likely reduction in insulin sensitivity accompanying obesity would be balanced by the positive effect of increased weight, but the negative effect on spine BMD of android fat would outweigh the effect of increased weight.

 The sequence of multivariable analyses we undertook constitute a mediation analysis, whereby the impact of introducing additional explanatory variables on regression coefficients and their significances can provide an indication of variables that could be further explored for a possible role in causation ([16](#_ENREF_16)). Accordingly, the positive associations we observed between regional fat mass and BMD were found to be secondary to the contribution fat mass makes to weight for, when variation of BMD with weight was taken into account, the associations between fat mass and BMD changed significantly and not only were the positive associations between fat mass and BMD eliminated, negative association between fat mass and BMD emerged. We then extended the mediation analysis to explore whether variation in insulin sensitivity might underlie those associations between fat or lean body masses and BMD that were independent of weight. When variation in BMD with Si was taken into account, these associations were eliminated and positive associations between Si and BMD were found. However, the role Si in modifying these associations is less certain as the regression coefficients did not change significantly with inclusion of Si. Moreover, the positive associations of Si with BMD were only apparent in those who reported exercising. Even light exercise can significantly increase insulin sensitivity ([19](#_ENREF_19)) and it is possible that the positive effect of Si on BMD we observed was associated with increased exercise. Our assessment of exercise was very simple and resolution of this issue will require studies with accurate, objective measures of exercise, for example by accelerometry.

 The independent, negative effect of fat mass on lumbar spine BMD, after adjustment for weight, has also been described in large cross-sectional studies ([7](#_ENREF_7), [20](#_ENREF_20)). Fat mass has also been found to be adversely associated with femoral neck BMD ([7](#_ENREF_7)) , hip BMD ([21](#_ENREF_21)) and total bone mineral content ([7](#_ENREF_7), [21](#_ENREF_21)). Our study extends these observations by identifying the independent negative associations of fat mass in the android region with spine BMD. Importantly, we established that the negative associations of android fat mass with spine BMD were independent of the other variables described. Attenuation of the DXA signal by android fat might have contributed to the independent negative associations of spine and rib BMD with android fat mass. However, this possibility has been discounted for the Lunar dual photon X-ray absorptiometer ([22](#_ENREF_22)) and the largest attenuation effect that has been detected with other instruments is smaller than the effect size we observed ([23](#_ENREF_23)). It has been suggested that higher visceral adiposity may be associated with higher bone marrow fat ([24](#_ENREF_24)) and an association of higher bone marrow fat with lower BMD ([25](#_ENREF_25)) has been reported. Further investigation of specific inflammatory cytokines or bone marrow fat in mediating the adverse relationship between central fat and spine BMD may be warranted.

 Lean mass has been reported to show a positive association with BMD, and its effect appears to be greater than that of fat mass ([1](#_ENREF_1)). We found lean mass to be positively associated with all measures of BMD and, in contrast to total and android fat mass, lean body mass eliminated the positive association between weight and spine BMD. To the extent that lean mass reflects muscle mass, lean mass has a crucial role in mechanical loading but, interestingly, we found that the positive effects of lean mass on BMD were independent of weight. This accords with the possibility of a muscle-bone endocrine crosstalk ([26](#_ENREF_26)).

 Our finding that insulin sensitivity was positively associated with total, leg and arm BMD is supported by two large studies which showed that after adjustment for weight or fat mass, the association of insulin resistance (using fasting insulin or HOMA-IR) with BMD changed in direction from positive to negative, and this was apparent for total, hip and femoral neck BMD ([4](#_ENREF_4), [27](#_ENREF_27)). However, in contrast to our finding of no association in predominantly middle-aged males, Choo et al, studying peak bone mass in young adults, found that increasing insulin sensitivity was positively associated with lumbar spine BMD ([27](#_ENREF_27)). Further studies will be needed to resolve whether variation in exercise might underlie this association. Nevertheless, our finding that insulin sensitivity is associated with increased limb BMD appears to be in keeping with the observation that the fracture rate for hip in patients with type 2 diabetes (T2DM) is greater than for other sites ([28](#_ENREF_28), [29](#_ENREF_29)). Mechanistic evidence for a positive association between BMD and insulin sensitivity comes from a report that in mice fed a high fat diet, insulin resistance impaired osteoblast survival and proliferation ([30](#_ENREF_30)). A further possible dimension to associations between insulin sensitivity and bone metabolism could involve interleukin-6 and tumor necrosis factor alpha since both can induce insulin resistance, are elevated in insulin resistant states and, as mentioned, can induce osteoclast mediated bone loss ([31](#_ENREF_31)). Overall though, it should also be acknowledged that there is only a limited correlation between BMD and fracture risk ([32](#_ENREF_32)).

Our study adds to the current limited understanding of interrelationships between insulin sensitivity, fat and BMD and challenges two common assumptions regarding fat, insulin sensitivity and BMD. Firstly, because obesity has been associated with increased BMD and reduced risk of fracture (2), it may be misconceived that fat mass itself would benefit BMD. We found some evidence for this but android fat had a negative effect on spine BMD that outweighed its mechanical loading benefit and this was independent of insulin sensitivity. Reduction of fracture risk associated with obesity is possibly related to its shock-absorbing effect (34). Secondly, while there has been evidence for insulin resistance having an adverse effect on total, hip and femoral neck BMD (4,29), this may not be a global effect. Our study showed that insulin sensitivity is positively associated with total, leg and arm BMD, but not with other regional BMDs, including spine. Further studies will need to elucidate the mechanisms underlying these differential associations.

Our study was limited in that it was cross-sectional with no intervention component and could not, therefore, indicate causal roles for any of the variables measured. Nevertheless, our observational findings suggest that longitudinal studies of regional BMD, with interventions to modify central adiposity or insulin sensitivity or both, would be of value. Insulin sensitivity measurements were only available in a subgroup, although subgroup characteristics and relationships were very similar to those for the whole group (not shown). We have no information on the relationships between BMD and fracture rates in our cohort. Our participants represented a restricted population so are not generalisable. It is also noteworthy that no participants with T2DM were included in our analysis, which may have reduced confounding by defective beta cell function, comorbidities and medication use. Measurements of additional covariates such as oestradiol, testosterone and inflammatory markers that can affect or be affected by adiposity, insulin sensitivity and bone density could have helped clarify mechanisms underlying the associations we observed ([31](#_ENREF_31), [33](#_ENREF_33)). We may, nevertheless, conclude that android fat exhibited an inverse relationship with spine BMD which was not related to the low insulin sensitivity associated with increased android fat. Insulin sensitivity was positively associated with limb BMD independently of fat mass and weight but a role for exercise in these associations remains a possibility.

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**Authors’ roles**

Study design: WL and IFG. Study conduct: JCS, IFG. Data collection: IFG. Data analysis: WL and IFG. Data interpretation: WL, JCS and IFG. Drafting manuscript: WL. Revising manuscript content: JCS, and IFG. Approving final version of manuscript: WJL, JCS and IFG. IFG takes responsibility for the integrity of the data analysis.

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Disclosures: the authors have nothing to declare

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Table 1.Baseline characteristics of all participants (n=590). Median (interquartile range) are shown for continuous variables and % (n) for categorical variables

|  |  |
| --- | --- |
|  | **Median (IQR) or****% (n)** |
| Age (years) | 50.4 (43.7,58.6) |
| BMI (kg/m2) | 25.7(24.1,27.8) |
| Weight (kg) | 81(74.1,89.9) |
| Smoking |  |
| Non-smokerLight smokingHeavy smoking | 84(495)11(67)5(26) |
| Alcohol |  |
|  Non-drinkerLight alcohol intakeHeavy alcohol intake- | 4(21)74(436)22(133) |
| Exercise |  |
| SedentaryModerateRegular | 47(276)32(191)21(123) |
| ***Insulin Sensitivity / Resistance*** |
| IVGTT-Si\* (/min/mU/L) | 3.2(2.3,4.5) |
| HOMA-IR\* | 2.3(1.5,3.3) |
| ***Body Composition***: mass |
| Total Fat (kg)  | 20.7(16.8,26.0) |
| Android Fat (kg) | 11.7(8.8,15.0) |
| Gynoid Fat (kg) | 6.1(5.0,7.6) |
| Lean Body (kg) | 56.5(52.6,60.6) |
|  |  |
| ***Body Composition***: percent body weight |
| Total Fat mass (% wt) | 25.5(21.9,29.8) |
| Android Fat (% wt) | 14.4(11.6,17.0) |
| Gynoid Fat (% wt) | 7.6(6.4,8.8) |
| Lean Body mass (% wt) | 69.5(65.2,72.9) |
| ***Bone Mineral Density*** (g/cm2) |
| Total | 1.29(1.17,1.28) |
| Leg | 1.36(1.28,1.42) |
| Arm | 1.02(0.96,1.10) |
| Pelvis | 1.19(1.12,1.28) |
| Lumbar Spine | 1.16(1.06,1.29) |
| Thoracic Spine | 1.05(0.97,1.16) |
| Rib | 0.76(0.72,0.81) |
| Head | 2.11(1.96,2.23) |

\* 274 participants who underwent an intravenous glucose tolerance test with measurement of plasma glucose and insulin concentrations

Table 2a: DXA TOTAL FAT MASS associations with bone mineral density. Multivariable regression coefficients (95%CI) are shown for relationships between bone mineral density in 8 different body regions and total fat mass in white males. Model 1 with co-variables: age, height, smoking, alcohol and exercise (n=590); Model 2 with body weight and co-variables (n=590); and Model 3 with body weight, IVGTT insulin sensitivity, Si, and co-variables (n=274). Regression coefficients may be taken as significant if the 95% CI range does not include zero. Significances for the difference between regression coefficients for total fat mass between Model 1 and Models 2 are shown as † p<0.05. †† p<0.01. ††† p<0.001 (there were no significant differences between Models 2 and 3). Standardised variables were entered throughout.

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
|  |  |  |  | **BONE MINERAL DENSITY** |  |  |  |
|  | **total** | **leg** | **arm** | **pelvis** | **lumbar spine** | **thoracic spine** | **rib** | **head** |
| **Weight plus co-variables** |
| **weight**  | 0.412(0.325,0.498) | 0.315(0.188,0.442) | 0.529(0.398,0.661) | 0.339(0.197,0.481) | 0.030(-0.116,0.176) | 0.248(0.094,0.389) | 0.582(0.450,0.714) | 0.304(0.154,0.455) |
| **Model 1: total fat mass plus co-variables** |
| **total fat**  | 0.265(0.187,0.343) | 0.181(0.102,0.260) | 0.294(0.216,0.373) | 0.219(0.136,0.295) | -0.139(-0.221,-0.058) | 0.048(-0.032,0.128) | 0.375(0.298,0.453) | 0.142(0.061,0.224) |
| **Model 2: total fat mass plus weight and co-variables** |
| **total fat**  | -0.192†††(-0.34,-0.040) | -0.166††(-0.322,-0.009) | -0.092††(-0.246,0.062) | -0.118†(-0.276,0.040) | -0.445††(-0.607,-0.283) | -0.421†††(-0.578,-0.264) | -0.231†††(-0.376,-0.086) | -0.212††(-0.374,-0.050) |
| **weight** | 0.603(0.429,0.777) | 0.458(0.278,0.637) | 0.510(0.334,0.687) | 0.441(0.260,0.622) | 0.403(0.218,0.589) | 0.619(0.439,0.799) | 0.801(0.634,0.967) | 0.468(0.282,0.654) |
| **Model 3: total fat mass plus weight, Si and co-variables** |
| **total fat** | -0.108(-0.313,0.097) | -0.108(-0.313,0.098) | 0.014(-0.200,0.227) | -0.022(-0.257,0.212) | -0.508(-0.741,-0.274) | -0.498(-0.736,-0.260) | -0.264(-0.481,-0.048) | -0.245(-0.493,0.004) |
| **weight** | 0.664(0.420,0.909) | 0.527(0.281,0.772) | 0.628(0.373,0.884) | 0.421(0.140,0.701) | 0.583(0.304,0.862) | 0.759(0.475,1.043) | 0.871(0.612,1.129) | 0.552(0.256,0.849) |
| **Si** | 0.175(0.059,0.291) | 0.198(0.082,0.315) | 0.229(0.108,0.350) | 0.117(-0.016,0.249) | 0.037(-0.095,0.169) | -0.014(-0.148,0.121) | 0.021(-0.101,0.144) | -0.019(-0.160,0.121) |

Table 2b: DXA ANDROID FAT MASS associations with bone mineral density - for details see legend to Table 2a

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
|  |  |  |  | **BONE MINERAL DENSITY** |  |  |  |
|  | **total** | **leg** | **arm** | **pelvis** | **lumbar spine** | **thoracic spine** | **rib** | **head** |
| **Model 1: android fat mass plus co-variables** |
| **android fat**  | 0.253(0.176,0.331) | 0.196(0.118,0.274) | 0.315(0.238,0.392) | 0.199(0.120,0.278) | -0.211(-0.290,-0.132) | -0.031(-0.111,0.049) | 0.348(0.271,0.425) | 0.088(0.006,0.169) |
| **Model 2: android fat mass plus weight and co-variables** |
| **android fat**  | -0.125†††(-0.257,0.007) | -0.039†(-0.175,0.097) | 0.053††(-0.080,0.187) | -0.098†(-0.235,0.039) | -0.574†††(-0.710,-0.437) | -0.542†††(-0.674,-0.410) | -0.183†††(-0.309,-0.057) | -0.299††(-0.438,-0.159) |
| **weight** | 0.532(0.379,0.685) | 0.330(0.172,0.488) | 0.368(0.213,0.523) | 0.417(0.258,0.576) | 0.508(0.351,0.666) | 0.718(0.564,0.871) | 0.746(0.599,0.892) | 0.543(0.381,0.703) |
| **Model 3: android fat mass plus weight, Si and co-variables** |
| **android fat** | -0.039(-0.219,0.141) | 0.041(-0.1390.221) | 0.167(-0.019,0.353) | -0.031(-0.236,0.175) | -0.641(-0.838,-0.444) | -0.644(-0.844,-0.444) | -0.217(-0.408,-0.027) | -0.346(-0.561,-0.131) |
| **weight** | 0.593(0.377,0.810) | 0.382(0.165,0.598) | 0.486(0.262,0.710) | 0.427(0.180,0.674) | 0.677(0.441,0.914) | 0.866(0.625,1.106) | 0.811(0.582,1.040) | 0.632(0.374,0.891) |
| **Si** | 0.182(0.065,0.299) | 0.218(0.100,0.335) | 0.253(0.132,0.375) | 0.114(-0.019,0.248) | -0.004(-0.133,0.124) | -0.057(-0.187,0.073) | 0.018(-0.106,0.142) | -0.045(-0.185,0.095) |

Table 2c: DXA GYNOID FAT MASS associations with bone mineral density - for details see legend to Table 2a

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
|  |  |  |  | **BONE MINERAL DENSITY** |  |  |  |
|  | **total** | **leg** | **arm** | **pelvis** | **lumbar spine** | **thoracic spine** | **rib** | **head** |
| **Model 1: gynoid fat mass plus co-variables** |
| **gynoid fat**  | 0.236(0.156,0.316) | 0.128(0.047,0.209) | 0.202(0.120,0.283) | 0.213(0.132,0.294) | 0.003(-0.080,0.086) | 0.143(0.061,0.224) | 0.354(0.275,0.433) | 0.197(0.114,0.279) |
| **Model 2: gynoid fat mass plus weight and co-variables** |
| **gynoid fat**  | -0.103††(-0.222,0.016) | -0.169††(-0.291,-0.047) | -0.198†††(-0.317,-0.079) | -0.015(-0.138,0.109) | 0.073(-0.057,0.202) | 0.018(-0.107,0.144) | -0.078†††(-0.192,0.036) | 0.056(-0.071,0.183) |
| **weight** | 0.501(0.366,0.635) | 0.438(0.301,0.576) | 0.589(0.455,0.724) | 0.336(0.197,0.476) | -0.103(-0.249,0.043) | 0.184(0.042,0.325) | 0.638(0.509,0.766) | 0.208(0.065,0.352) |
| **Model 3: gynoid fat mass plus weight, Si and co-variables** |
| **gynoid fat** | -0.084(-0.248,0.802) | -0.162(-0.326,0.002) | -0.220(-0.389,-0.051) | 0.054(-0.134,0.242) | 0.020(-0.173,0.214) | -0.021(-0.218,0.175) | -0.091(-0.266,0.085) | 0.059(-0.142,0.259) |
| **weight** | 0.630(0.433,0.826) | 0.560(0.364,0.756) | 0.832(0.630,1.035) | 0.352(0.126,0.577) | 0.060(-0.172,0.292) | 0.282(0.047,0.518) | 0.686(0.476,0.896) | 0.258(0.018,0.498) |
| **Si** | 0.187(0.073,0.300) | 0.209(0.096,0.322) | 0.224(0.107,0.341) | 0.120(-0.010,0.250) | 0.097(-0.036,0.231) | 0.045(-0.091,0.181) | 0.051(-0.070,0.172) | 0.011(-0.128,0.149) |

Table 2d: DXA TOTAL LEAN MASS associations with bone mineral density - for details see legend to Table 2a

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
|  |  |  |  | **BONE MINERAL DENSITY** |  |  |  |
|  | **total** | **leg** | **arm** | **pelvis** | **lumbar spine** | **thoracic spine** | **rib** | **head** |
| **Model 1: lean mass plus co-variables** |
| **lean** | 0.435(0.334,0.535) | 0.320(0.218,0.423) | 0.417(0.315,0.518) | 0.369(0.266,0.472) | 0.167(0.060,0.274) | 0.363(0.261,0.465) | 0.582(0.484,0.680) | 0.270(0.164,0.376) |
| **Model 2: lean mass plus weight and co-variables** |
| **lean**  | 0.219(0.089,0.350) | 0.176(0.041,0.311) | 0.179†(0.047,0.311) | 0.222(0.086,0.357) | 0.346(0.207,0.487) | 0.374(0.239,0.509) | 0.269†(0.145,0.393) | 0.136(-0.004,0.276) |
| **weight** | 0.286(0.172,0.400) | 0.192(0.074,0.310) | 0.316(0.201,0.431) | 0.196(0.078,0.314) | -0.239(-0.361,-0.117) | -0.015(-0.133,0.103) | 0.416(0.308,0.525) | 0.179(0.057,0.301) |
| **Model 3: lean mass plus weight, Si and co-variables** |
| **lean** | 0.180(0.016,0.343) | 0.188(0.025,0.352) | 0.060(-0.111,0.231) | 0.176(-0.011,0.363) | 0.311(0.121,0.501) | 0.387(0.196,0.578) | 0.304(0.132,0.476) | 0.192(-0.007,0.392) |
| **weight** | 0.455(0.292,0.619) | 0.313(0.149,0.477) | 0.608(0.436,0.779) | 0.299(0.111,0.486) | -0.098(-0.289,0.092) | 0.045(-0.147,0.236) | 0.436(0.263,0.608) | 0.200(0.000,0.400) |
| **Si** | 0.169(0.055,0.283) | 0.192(0.078,0.306) | 0.221(0.101,0.340) | 0.101(-0.029,0.232) | 0.065(-0.068,0.197) | 0.005(-0.128,0.138) | 0.021(-0.098,0.140) | -0.010(-0.149,0.129) |

Legend to Figure 1: In the HDDRISC study, obese participants were on average 9 kg heavier than normal weight participants with a predicted reduction in insulin sensitivity, Si, of 2.42 /min/mU/L. Shown here are the independent effects on regional BMD of a 9kg increase in android fat or weight and a 2.42 /min/mU/L decrease in Si, in terms of percent change in BMD from normal weight (BMI 20-25kg/m2) to obese (BMI≥30kg/m2). Effect sizes were derived from the coefficients for multivariable analysis Model 3, Table 2b, as were significances.



Supplementary Table 1

Associations of individual characteristics with body composition measures. Univariable regression coefficients (95%CI) are shown for all variables (n=590, except Si for which n=274) and multivariable coefficients are shown for weight with other variables included as explanatory variables in each model. All continuous variables were standardised except age.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | **total fat mass \*** | **android fat mass \*** | **gynoid fat mass\*** | **lean body mass\*** |
| UNIVARIABLE |  |  |  |  |
| **Weight\*** | 0.830**\*\*\***(0.785,0.875) | 0.775**\*\*\***(0.724,0.826) | 0.770**\*\*\***(0.719,0.822) | 0.759**\*\*\***(0.708,0.811) |
| **Si\*** | -0.451**\*\*\***(-0.559,-0.343) | -0.463**\*\*\***(-0.570,-0.355) | -0.314**\*\*\***(-0.424,-0.204) | -0.126**\***(-0.243,-0.010) |
| **Height\*** | -0.001(-0.009,0.006) | 0.001(-0.007,0.008) | -0.008**\***(-0.015,-0.001) | -0.015**\*\*\***(-0.022,-0.007) |
| **Age (years)** | -0.203**\*\*\***(-0.283,-0.123) | -0.159**\*\*\***(-0.240,-0.079) | -0.262**\*\*\***(-0.341,-0.183) | -0.660**\*\*\***(-0.721,-0.599) |
| **Light smoking** | -0.050(-0.305,0.205) | -0.075(-0.330,0.180) | -0.024(-0.279,0.232) | 0.079(-0.175,0.333) |
| **Heavy smoking** | 0.484**\***(0.089,0.878) | 0.434**\***(0.040,0.828) | 0.443 **\***(0.048,0.838) | 0.109(-0.283,0.502) |
| **Light alcohol****Intake** | 0.216(-0.223,0.655) | 0.165(-0.274,0.604) | 0.202(-0.237,0.642) | 0.320(-0.112,0.753) |
| **Heavy alcohol****Intake** | 0.267(-0.194,0.729) | 0.199(-0.262,0.660) | 0.247(-0.215,0.709) | 0.559(0.105,1.014) |
| **Moderate exercise** | -0.333**\*\*\***(-0.505,-0.160) | -0.311**\*\*\***(-0.483,-0.139) | -0.316**\*\*\***(-0.489,-0.144) | -0.001(-0.174,0.172) |
| **Regular exercise** | -0.315**\***(-0.563,-0.068) | -0.281**\***(-0.529,-0.033) | -0.282 **\***(-0.530,-0.034) | 0.094(-0.155,0.342) |
| MULTIVARIABLE |  |  |  |  |
| **Weight\***† | 0.997**\*\*\***(0.951,1.044) | 0.957**\*\*\***(0.903,1.010) | 0.865**\*\*\***(0.805,0.925) | 0.573 **\*\*\***(0.519,0.626) |
| **Weight\***§ | 0.993**\*\*\***(0.912,1.074) | 0.934**\*\*\***(0.842,1.026) | 0.866**\*\*\***(0.766,0.967) | 0.568**\*\*\***(0.467,0.668) |
| **Si\***§ | -0.119**\*\*\***(-0.187,-0.052) | -0.159**\*\*\***(-0.236,-0.083) | -0.014(-0.098,0.070) | 0.105**\***(0.021,0.189) |

\* p<0.05. \*\* p<0.01. \*\*\* p<0.001. † without Si (n=274). § with Si (n=274).