A METABOLIC SCREEN IN ADOLESCENTS REVEALS AN ASSOCIATION BETWEEN CIRCULATING CITRATE AND CORTICAL BONE MINERAL DENSITY

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DISCLOSURE

All authors state that they have no conflicts of interest.

ABSTRACT

Observations that insulin and adiponectin levels are related to cortical bone size in adolescents, independently of body composition, suggest factors related to fat metabolism directly influence skeletal development. To explore this question, we examined associations between a metabolic screen focusing on fat metabolism, and peripheral quantitative computed tomography (pQCT) measures of the mid-tibia, in 15 year-olds from the Avon Longitudinal Study of Parents and Children. Metabolic profiles were generated by proton nuclear magnetic resonance spectroscopy, from blood samples obtained at the same time as pQCT scans. Ordinary least squares linear regression was used to investigate relationships between metabolic measures and periosteal circumference (PC), cortical thickness (CT) and cortical (BMDC). Metabolic profiles yielded 22 independent components following PCA, giving a Bonferroni-adjusted threshold for statistical significance of P=0.002. Data were available in 1121 subjects (487 males), mean age 15 years. Several metabolites related to lipid and cholesterol metabolism were associated with PC, CT and BMDC after adjustment for age, sex and Tanner stage. After additional adjustment for height, fat and lean mass, only the association between citrate and BMDC remained below the Bonferroni-significant threshold [β=−0.14 (−0.18,−0.09)] (β represents a standardised coefficient). Citrate also showed evidence of association with periosteal circumference [PC, β=0.06 (0.03,0.10)] and strength strain index [SSI, β=0.04 (0.01,0.08)]. Subsequently, we investigated whether these relationships were explained by increased bone resorption. Citrate was strongly related to serum β-C-telopeptides of type I collagen (β-CTX) [β=0.20 (0.16,0.23)]. After additional adjustment for β-CTX the above associations between citrate and BMDC [β=−0.04 (−0.08,0.01)], PC [β=0.03 (-0.01,0.07)] and SSI [β=0.03 (-0.01,0.07)] were no longer observed. We conclude that in adolescents, circulating levels of citrate are inversely related to BMDC and positively related to PC, reflecting associations with higher bone turnover. Further studies are justified to elucidate possible contributions of citrate, a constituent of bone matrix, to bone resorption and cortical density.

Five key words: pQCT; bone resorption; β-CTX; lipids; ALSPAC

INTRODUCTION

Acquisition of peak bone mass is influenced by a range of constitutional factors, identification of which may provide new opportunities to optimise this process and reduce the risk of osteoporotic fractures in late life. For example, whereas lean mass is strongly related to bone mass and skeletal development, fat mass is also thought to play an important independent role [1]. In a previous study of adolescents from the Avon Longitudinal Study of Parents and Children (ALSPAC cohort), we used tibial pQCT to establish independent relationships between lean mass, fat mass and cortical bone size and density[2]. Whereas positive relationships between fat mass and bone parameters could reflect a response to greater mechanical strain caused by greater weight, we previously observed fat mass to be positively related to upper limb bone mass, suggesting a role of metabolic pathways independently of body weight [1]. In terms of metabolic pathways which might mediate positive influences of fat mass on bone accrual, we previously found that whereas fat mass is inversely related to adiponectin levels, the latter is inversely related to cortical bone size [3].

The availability of metabolic screens provides an opportunity to identify additional influences on bone development by examining multiple factors simultaneously. For example, having related results from a metabolic screen of 280 known metabolites to bone measures in older individuals, Moayyeri et al identified several new metabolites related to BMD [4]. In order to extend understanding of the relationship between fat metabolism and bone development, we utilised a metabolic platform to quantify a range of measures related to fat metabolism in ALSPAC [5,6]. Here we report associations between results from this platform and cortical bone size and density, obtained by pQCT of the mid-tibia in 15 year-olds from ALSPAC. To identify potential mechanistic pathways acting directly on the skeleton, as opposed to indirectly via altered body composition, additional models were analysed following adjustment for fat and lean mass.

MATERIALS AND METHODS

The Avon Longitudinal Study of Parents and Children (ALSPAC) is a population-based birth cohort comprising 14,541 children born between 1 April 1991 and 31 December 1992 and their mothers from the county of Avon, UK. Full study methodology is described elsewhere [7,8] and the ALSPAC website contains details of all the data that are available through a fully searchable data dictionary (http://www.bris.ac.uk/alspac/researchers/data-access/data-dictionary/). The present study is based on research clinics to which the whole cohort was invited and held when participants were a mean age of 15.5 years (supplementary Figure 1). Parental consent and child's consent was obtained for all measurements made. Ethical approval for the study was obtained from the ALSPAC Ethics and Law Committee and from the UK National Health Service Local Research Ethics Committees.

Tibial pQCT

Cortical bone mineral density (BMDc) and bone mineral content (BMCc) of the mid (50% from the distal endplate) right tibia were obtained using a Stratec XCT2000L in conjunction with XCT 2000 imaging software version 6.00. (Stratec, Pforzheim, Germany) during the teen focus three (TF3, mean age = 15.5 years) research clinic to which all ALSPAC participants were invited as part of a study investigating the effects of physical activity on cortical bone as previously published [3]. Periosteal circumference (PC), endosteal circumference (EC) and cortical thickness (CT) were derived using a circular ring model as described in the Stratec user manual. Cortical bone was defined using a threshold above 650 mg/cm^3 [3], and cortical bone mineral density (BMDc) subsequently derived. Strength strain index (SSI) was calculated according to the formula published by Hasegawa et al [9]. Within Subject coefficient of variation for pQCT parameters are displayed in parentheses: tibial length (4.04%), BMCc (2.71%), BMDc (1.29%), PC (1.58%), EC (4.03%), SSI (3.72%). For the purposes of the present study, PC, CT and BMDc were used as separate outcomes, on the basis that they reflect distinct and largely independent measures of cortical bone size (PC, CT) or density (BMDc).

Metabolic Screen

A high-throughput serum nuclear magnetic resonance metabolic platform was used to quantify metabolic measures focusing on fat metabolism [5,6], based on fasting serum samples taken at the TF3 clinic. Details of the experimentation have been described elsewhere [6,10]. In brief, these measures comprise ketone bodies, glycolysis related metabolites, inflammation, fatty acids, amino acids, cholesterol, apolipoproteins, measures of fluid balance, glycerides and

phospholipids. Cholesterol (total, free and esterified) and triglycerides were measured as total levels and/or associated with one of 14 lipoprotein subclasses (6 VLDL, IDL, 3 LDL and 4HDL). Several ratios between different metabolites were also derived. Though 230 measures were obtained in total, analyses using this screen generally focus on a sub-sample of 73 representative variables classified into thirteen classes [11], (see Figure 1). Due to the correlated nature of the metabolites, we defined our multiple testing threshold for metabolite wide association analysis by principal component analysis (PCA) as previously described [12]. Briefly, PCA is a mathematical data reduction tool that transforms a set of possibly correlated variables into a set of linearly uncorrelated variables (i.e. principal components). The transformation is defined such that the first component explains most of the variation in the sample, and each subsequent component explains the largest amount of variance possible, under the constraint that it is uncorrelated all preceding components. In this study, PCA estimated that 22 components explained > 95% of the variance of all 230 metabolites measured, and in the smaller set of 73 representative metabolites. Consequently, a type-1 error rate of $\alpha = 0.002$ (0.05 / 22 components) was used to infer statistical significance for the metabolite screen.

Other variables

Height was measured using a Harpenden stadiometer (Holtain Ltd., Crymych, UK) and weight was measured to the nearest 50 g using Tanita weighing scales (Tanita UK Ltd, Uxbridge). Data on lean mass and fat mass were obtained from total body DXA scans performed at the TF3 clinic, using a Lunar Prodigy scanner and imaging software [version 10.10.038 (Lunar Radiation Corp, Madison, WI)]. In view of the important influence of time since age of peak height velocity on pQCT parameters [13], we aimed to account for time since puberty in this largely post pubertal cohort. Therefore, we adjusted for age of puberty onset, based on results for Tanner stage at age 13.5 years as assessed by questionnaire (pubic hair domain), as previously found to be related to hip development as assessed by DXA [14]. Electrochemiluminescence immunoassays (ECLIA) (Roche Diagnostics, Lewes, UK) were used to measure plasma concentrations of serum β-C-telopeptides of type I collagen (β-CTX) on fasting samples collected at the TF3 clinic visit (detection limit 0.01 ng/ml), plasma being separated and frozen within four hours at −80ºC. Inter- and intra-assay coefficients of variation (CVs) were <6.0% across the working range.

Statistical Analysis

Prior to analysis, all outcome variables were visually inspected to ensure they were normally distributed. pQCT bone outcome data and metabolic exposures, the combined sample of males and females were standardized to z-scores with mean = 0, standard deviation = 1 by subtracting individual measures from the sample mean, and dividing each value by the sample standard deviation. Ordinary least squares (OLS) linear regression was used to investigate: (i) the relationship between each metabolic measure and PC, CT and BMDC adjusted for age and sex (i.e. model 1), (ii) the effect of further adjustment for Tanner stage (model 2), (iii) the effect of further adjustment for height (model 3), and (iv) for lean mass, fat mass and height (i.e. model 4). Fat and lean mass were adjusted for, in preference to weight, to account for the distinct relationship of these two compartments with cortical bone parameters [3]. A type-1 error rate of $\alpha = 0.002$ (0.05/22) was used to infer statistical significance for each of the three traits examined, following Bonferroni correction to account for the number of independent metabolic traits identified by PCA. Sex differences were explored by comparing standardised β coefficients (expressed as SD change in outcome per SD change in metabolite) between separate analyses in males and females and by testing for sex interactions in analyses performed in males and females combined. Finally, based on a hypothesis that i) lower BMDC reflects greater cortical porosity due to higher bone turnover and ii) higher bone turnover leads to greater periosteal expansion and strength [15]; we investigated if the relationship between citrate and BMDC that we observed was explained by increased bone turnover, by further adjusting model 4 for β-C-telopeptides of type I collagen (β-CTX) and time of clinic attendance [whether participants attended a morning or afternoon clinic, to take account of diurnal variation in β-CTX (samples largely clustered to within an hour of 8 a.m. or 12 p.m.)]. Note: When used as an outcome variable, β-CTX was log transformed to normality and standardised to z-scores prior to regression analysis. All regression models involving β-CTX were adjusted for time of clinic attendance in addition to model specific covariates.

RESULTS

Description of participants

1121 participants (634 females and 487 males) were identified in ALSPAC who had valid measurements for serum metabolites, anthropometry, pQCT and DXA data generated at the TF3 clinic, and with Tanner Stage derived during the TF2 clinic (supplementary Figure 1). Descriptive statistics are presented in Table 1. Briefly, height, weight, lean mass and citrate and β-CTX were greater in males, in contrast to fat mass, which was substantially greater in female participants. BA_C, BMC_C, CT, PC, EC and SSI were larger in male participants, whereas BMD_C was larger in females.

Metabolic screen

Periosteal Circumference: In our minimally adjusted model (i.e. model 1), metabolites from the following categories were inversely associated with PC: lipoprotein concentrations, lipoprotein particle size, apolipoproteins, triglycerides, phospholipids, cholesterol and fatty acids (supplementary Figure 2). In contrast, creatinine and the amino acid phenylalanine were positively related to PC. The above-mentioned associations remained largely unchanged after correction for Tanner stage (model 2). Further adjustment for height resulted in the partial attenuation of several metabolites from each of the following classes: lipoprotein concentrations, triglycerides, phospholipids and fatty acids (model 3). Additional adjustment for body composition (model 4) resulted in the attenuation of all remaining relationships, except for citrate, for which there was now weak evidence of a positive association (see below).

Cortical thickness: Lipoproteins, lipoprotein particle size, apolipoproteins, triglycerides, phospholipids, cholesterol, fatty acids and citrate were inversely associated with CT in model 1 (supplementary Figure 3). In contrast, amino acids leucine and histidine were positively related to CT. Associations remained largely unchanged in model 2, except for citrate, which was attenuated towards the null. In model 3, associations between metabolites from the following classes were partially attenuated: lipoprotein concentrations, triglycerides, phospholipids, cholesterol, fatty acids and amino acids leucine and histidine. Further adjustment for lean and fat mass resulted in the attenuation of all remaining associations (model 4).

Cortical bone mineral density: In model 1, lipoproteins, apolipoproteins, triglycerides, phospholipids, cholesterol, fatty acids, glucose and citrate were inversely associated with

BMDC (Figure 1) (see Figure 2A for citrate versus BMDC scatterplot in males and females). Effect sizes were approximately β=−0.1, with the exception of the association with citrate where β=−0.21 (Figure 1 and supplementary Table 1). In contrast, creatinine and albumin were positively related to BMDC. In model 2, partial attenuation of several associations was seen, and apolipoproteins, triglycerides, glucose and albumin were no longer robustly associated with BMDC. In model 3, associations between the remaining various lipoprotein concentrations, LDL cholesterol, monounsaturated fatty acids and creatinine were partially attenuated, whereas the association between total, esterified and free cholesterol, and citrate were observed. For model 4, the beta coefficients were largely unchanged, and there was evidence of association in the case of lipoprotein concentrations, phospholipids, cholesterol, fatty acids, creatinine and citrate, however P-values only remained below the Bonferroni-adjusted cut-off for citrate ($P=2.4x10^{-10}$). The relationship between BMDC and citrate appeared linear. Specifically, a Wald test comparing regression model 1 to model 1 + $Citrate^2$ suggested that both models were similar (P=0.66) and the addition of the quadratic term did not significantly improve model fit. Similar results were obtained for BMDC and other bone outcomes when adjusting for all covariates and/or when modelling an additional cubic term.

Follow-up of citrate associations

Citrate versus pQCT parameters: Further analyses were performed to follow up on the finding that citrate was the only metabolite to show strong evidence of association in our fully adjusted model. First, we examined associations between citrate and all pQCT parameters (see Table 2). In model 4, positive associations were observed between citrate and: periosteal circumference [PC, β= 0.06 (0.03, 0.10)], strength strain index [SSI, β= 0.04 (0.01, 0.08)] and endosteal circumference [EC, β= 0.07 (0.01, 0.13)] (Table 2). Analysis of sex interaction as per model 4 revealed that the association between citrate on BMDC was larger in males [(β_{males}= −0.18 (−0.26, −0.11)] when compared with females [($\beta_{females}$= −0.07 (−0.12, −0.02)] ($P_{SexInt} = 3\times10^{-5}$). No robust evidence of a sex interaction was detected between citrate and PC (P_{SexInt} = 0.18) or SSI (P_{SexInt} = 0.44), or EC (P_{SexInt}=0.48).

Serum citrate versus β-CTX: Having previously observed an equivalent divergent association with BMDC and PC in the case of β-CTX [15], we hypothesized that relationships between citrate and pQCT parameters described above also involve altered levels of bone resorption. Scatterplots in males and females of β-CTX versus BMDC, and serum citrate versus β-CTX,

are presented in Figures 2B and 2C respectively. In adjusted analyses, we observed a strong association between citrate and β-CTX in all four models [e.g. β= 0.20 (0.16, 0.23), model 4] (see Table 2). Although beta coefficients appeared slightly larger in males [(β_{males}= 0.22 (0.16, 0.27)] compared to females [($\beta_{females}$= 0.18 (0.13, 0.23)], no robust evidence of a sex interaction was observed (P_{SexInt}=0.23).

Adjustment of citrate versus cortical bone mineral density for β-CTX: To examine whether associations between citrate and BMDC and other pQCT parameters involve altered levels of β-CTX, the above associations were re-analysed following additional adjustment for β-CTX. In both males, females, and both sexes combined, associations between citrate and BMDC were attenuated when β-CTX adjustment was added to model 4 (Figure 3), as were associations in sex combined analyses with PC [β = 0.03 (−0.01, 0.07)] and SSI [β = 0.03 (−0.01, 0.07)].

Age of puberty onset versus citrate: To examine why associations between citrate and cortical thickness and BMDC showed evidence of attenuation following adjustment for Tanner stage at age 13.5, we examined relationships between the latter and citrate levels. As shown in supplementary Figure 4, citrate levels appeared to decline with increasing time since puberty.

DISCUSSION

We report associations between a metabolic screen focused on fat metabolism, and cortical bone parameters of the mid-tibia measured by pQCT, in a population-based cohort of adolescents. Whereas many categories of lipid metabolites were associated with PC, CT and BMDC in minimally adjusted models, most associations were attenuated following adjustment for fat and lean mass. That said, several lipoproteins, phospholipids, cholesterol and fatty acids, were at least nominally associated with BMDC in our fully adjusted model, with inverse associations consistently observed. In addition, we observed an inverse association between citrate levels and BMDC, including in our fully adjusted model, which was considerably stronger than associations seen with lipid metabolites.

It is well established that citrate is present in significant quantities in bone, likely originating from osteoblasts [16], where it has been suggested to play a role in stabilising apatite nanocrystals [17], and forming bridges between mineral platelets [18]. Approximately 90% of citrate resides in bone, from which its release is suggested to be the principle determinant of plasma citrate levels, in line with previous studies which suggest that PTH administration leads to an increase in plasma citrate [19]. Our finding that citrate levels were strongly related to the resorption marker, β-CTX, is in line with this view that circulating levels of citrate are derived from bone breakdown.

Our observation that the inverse association between citrate and BMDC was no longer observed after adjustment for β-CTX raises the possibility that the inverse association we found between citrate and BMDC is mediated by increased bone resorption. This may explain, why the association between citrate levels and BMDC was stronger in boys compared to girls, since at age 15, boys are closer to their pubertal growth spurt compared to girls, and consequently likely to be undergoing considerably higher rates of bone modelling and remodelling [20]. In addition, a positive association between citrate levels and bone resorption would explain the positive association between citrate and PC, in view of our previous observations in the same cohort which suggest that bone resorption provides a stimulus for periosteal expansion [15].

Given the observational nature of our analysis, we are unable to determine the causal nature of the relationships we observed between citrate levels, bone resorption as reflected by β-CTX, and BMDC. It is well recognised that greater bone resorption leads to reduced BMD. However

the inverse association between circulating citrate levels and BMDc might reflect not only a positive effect of bone resorption on circulating citrate levels, but also a positive effect of circulating citrate levels on bone resorption. For example, circulating citrate levels are influenced by a number of other factors including diet and renal excretion [19], which are themselves likely to influence citrate levels within bone, of which the latter could theoretically affect bone resorption by controlling the solubility of bone mineral (personal communication, Melinda Duer). To the extent that citrate levels are determined by bone resorption, citrate levels may reflect degradation of bone mineral as opposed to type I collagen, providing additional information about bone resorption over and above that obtained through measurement of β-CTX alone.

While the present study was based on adolescents, it's currently unclear whether the same conclusions apply to other age groups. In a study published recently, citrate levels were found to be considerably higher in young (mean age 18.8) versus older (mean age 64.5) males, which is expected given bone turnover (as reflected by β-CTX) was over two-fold higher in the former group [21]. However, in a further analysis based on 87 older mixed subjects, there was only weak evidence of an inverse association between serum citrate and turnover markers including β-CTX [21]. Moreover, the authors found citrate to be positively related to both lumbar spine and hip BMD in this group. Although this may suggest that the relationship between citrate and BMD reported here is modified with age, it should be noted that in contrast to BMDc, DXA-derived BMD represents an 'areal' density, and as such is affected by skeletal size as reflected PC, which we found to be positively related to citrate levels.

Whereas weaker associations were observed between fat metabolites and BMDc compared to those seen with citrate, lipoproteins, phospholipids, cholesterol and fatty acids all showed evidence of inverse associations including in our fully adjusted model. These findings raise the intriguing possibility that fat metabolism exerts a direct influence on skeletal development. That lower levels of fat metabolites are associated with higher BMD is consistent with findings from a recent meta-analysis that cholesterol lowering agents, statins, improve BMD as measured by DXA and reduce fracture risk [22].

Limitations

These analyses were only performed on a subgroup of ALSPAC participants, which may have differed in certain ways from the original cohort, however this is only likely to have introduced spurious associations if these characteristics are related in different ways to pQCT and metabolic results which seems unlikely. As with all cross sectional studies, our analyses are potentially limited by confounding. This may have applied particularly to findings concerning relationships with lipid metabolites, which may be influenced by a range of lifestyle and socio-economic factors which are also related to measures of skeletal health, such as physical activity. Finally, since Tanner stage data was not available at age 15 years, we were unable to adjust our results for current pubertal stage.

Conclusions

We examined associations between a metabolic screen focused on fat metabolism and cortical bone parameters in adolescents. Several lipoproteins, phospholipids, cholesterol and fatty acids showed evidence of inverse associations with BMDc in our fully adjusted model, consistent with the known protective effect of lipid lowering agents on BMD as measured by DXA. In addition, we observed a novel inverse association between citrate levels and BMDC, including in our fully adjusted model, which was considerably stronger than associations seen with fat metabolites. Further analyses revealed that citrate is also strongly positively related to bone resorption as reflected by β-CTX, and that when this was adjusted for, the relationship between citrate and BMDC is no longer observed. Based on these observations, further analyses are justified to explore whether citrate might prove useful as a biomarker of bone resorption, by reflecting the removal of bone mineral as opposed to type I collagen.

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FIGURE LEGENDS

Figure 1: Summary of relationships between 73 representative serum metabolites and pQCT derived cortical bone mineral density measured at the tibia. Outcome and exposure measures were standardised prior to analysis (mean = 0 and standard deviation = 1). Cortical bone mineral density [BMDC (outcome)] was regressed on 73 representative metabolites (exposure) correcting for age and sex (model 1). Stepwise regression involved incorporating additional covariates, namely Tanner stage (model 2), height (model 3), and lean mass and fat mass (model 4). A Bonferroni multiple testing threshold of $P < 0.002$ (0.05 / 22 principal components) was used to identify metabolites that were robustly associated with BMDC. Note: Point estimates of β are expressed as SD change in outcome per SD change in metabolite and denoted by circles with corresponding 95% CIs denoted by bars. Observations with sufficient strength of evidence to reject the null hypothesis of no association between the metabolite and CT are coloured in black and their corresponding names highlighted in black. Observations not meeting the significance threshold are coloured in grey. Metabolites are classified into 13 categories, namely: Inflammation, Lipoprotein concentration, Particle size, Apolipoproteins, Triglycerides, Phospholipids, Cholesterol, Fatty acids, Fatty acid ratios, Fluid balance, Amino acids, Glycolosis related and Ketone bodies. VLDL = very low density lipoprotein, LDL = low density lipoprotein, IDL = intermediate density lipoprotein, HDL = high density lipoprotein, TG = triglycerides, C = cholesterol and FA = Fatty acids

Figure 2: Bivariate scatter plots describing the unadjusted relationships between citrate, pQCT derived cortical bone mineral density measured at the tibia (BMDC), and β-C-telopeptides of type I collagen (β-CTX). Data points are colored according to sex (males = dark grey and females = light grey). Panel A = serum citrate (x-axis) vs BMDC (y-axis); panel B = β-CTX (x-axis) vs BMDC (y-axis), and panel C = serum citrate (x-axis) vs β-CTX (y-axis).

Figure 3: Evaluating the relationship between citrate and cortical bone mineral density before after adjusting for β-CTX. Associations between citrate and BMDC (model 4) were attenuated after adjusting for β-CTX in the combined sample of males and females [(β_{comb}= −0.04 (−0.08, 0.01)], and the female-only [($\beta_{females}$= −0.01 (−0.06, 0.04)] and male only samples [(β_{males}= −0.06 (−0.13, 0.01)]. Point estimates of β are denoted by circles and expressed as SD change in BMDC per SD change in serum citrate. Bars denote corresponding 95% CIs.

FIGURES:

Figure 1

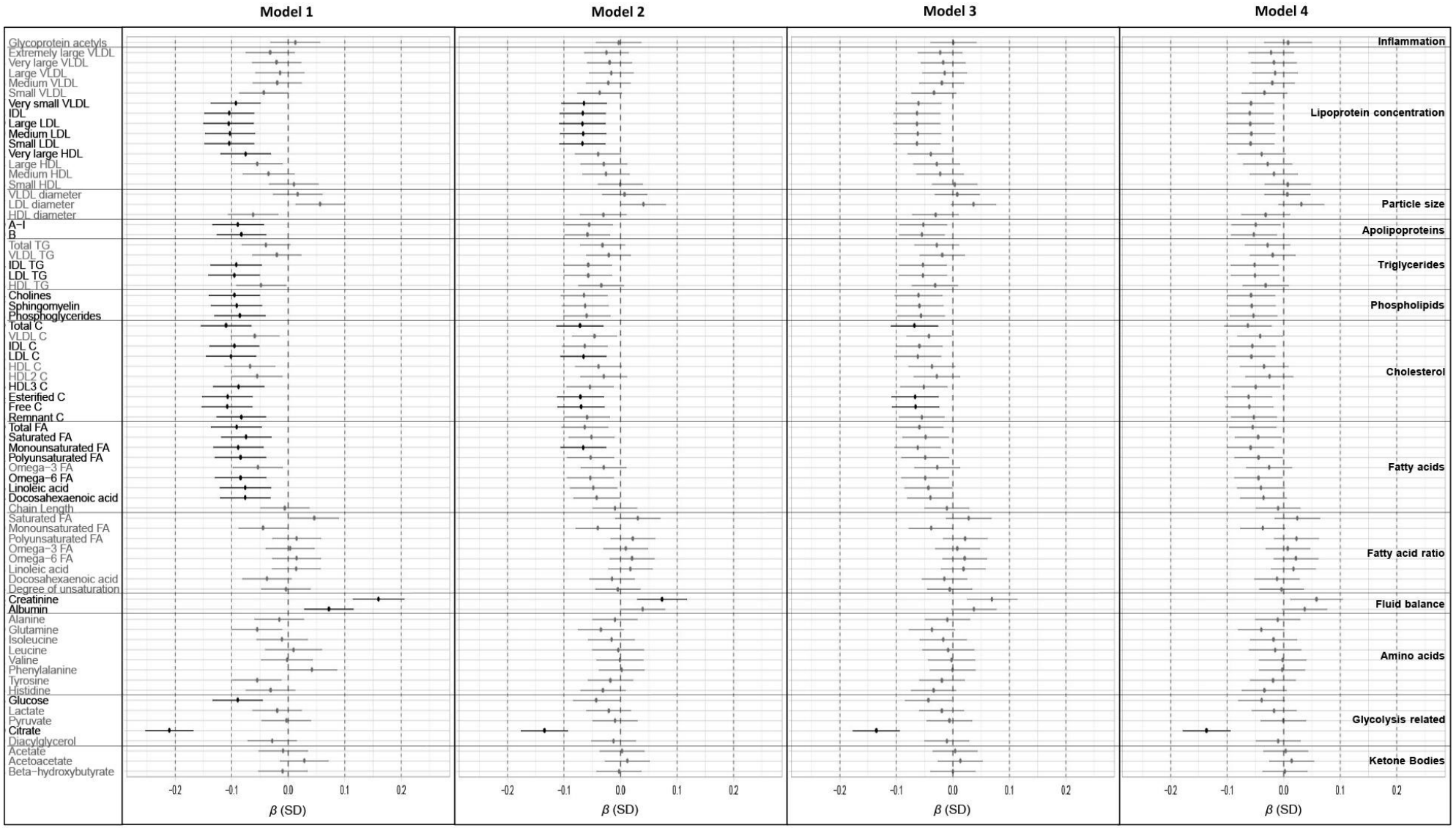

Figure 2.

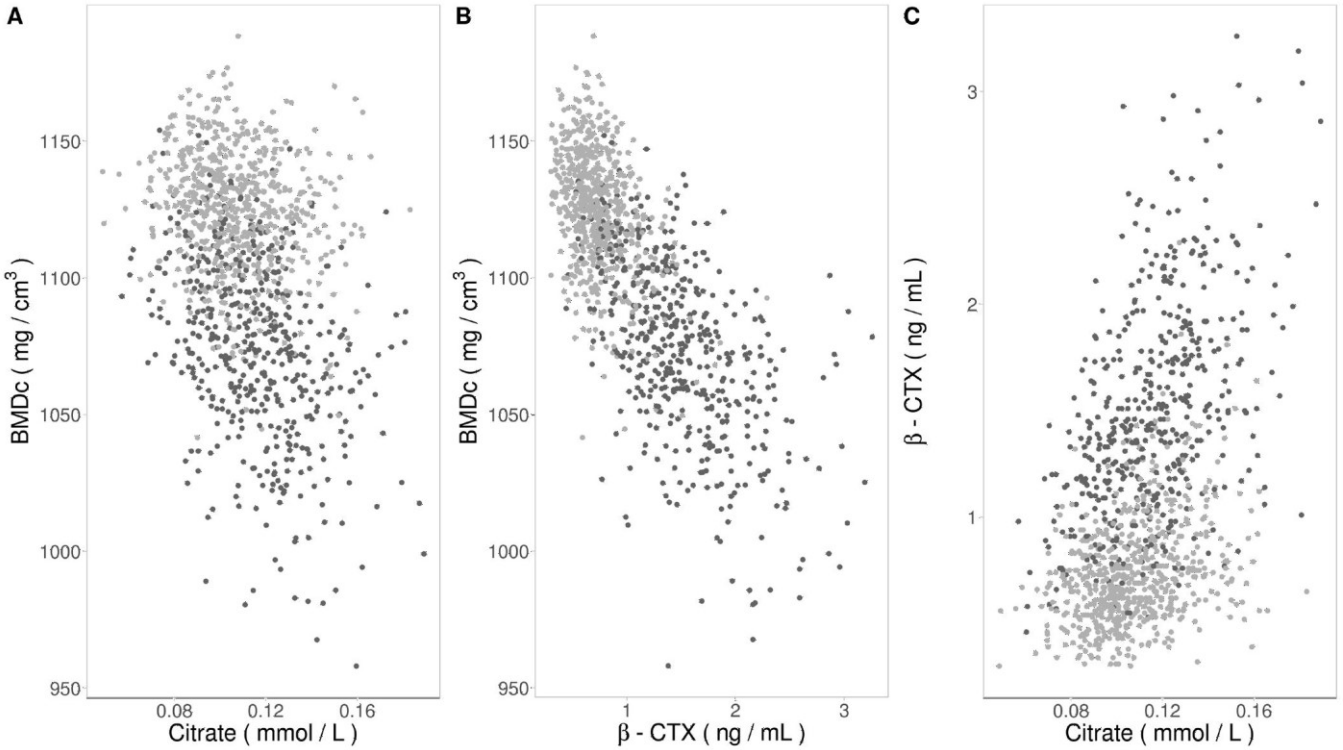
A
B
C
BMDc (mg / cm3)
1150
1100
1050
1000
950
0.08
0.12
0.16
Citrate (mmol / L)
β - CTX (ng / mL)
1
2
3

Figure 3

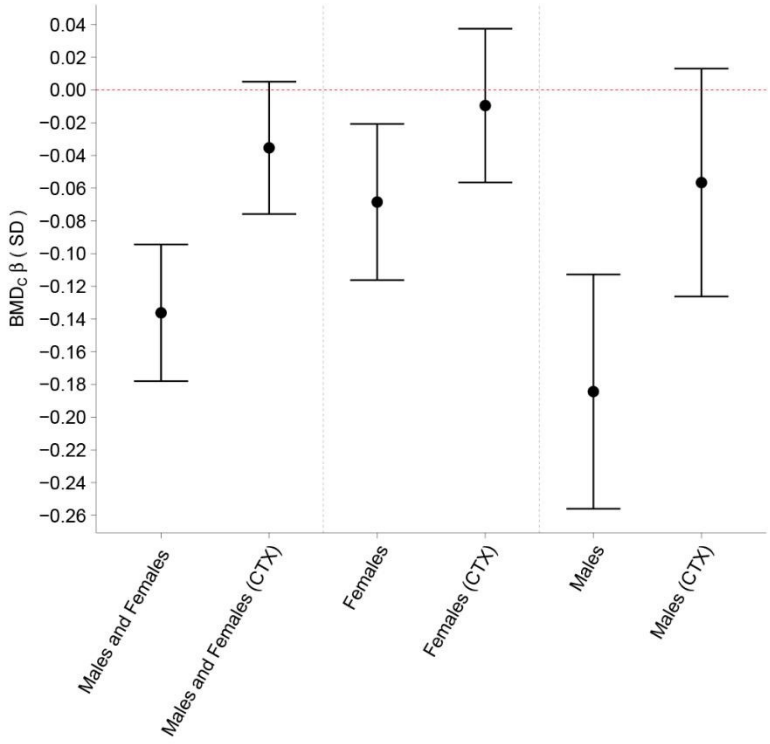
0.04
0.02
0.00
−0.02
−0.04
−0.06
−0.08
−0.10
−0.12
−0.14
−0.16
−0.18
−0.20
−0.22
−0.24
−0.26
BMDc β (SD)
Males and Females
Males and Females (CTX)
Females
Females (CTX)
Males
Males (CTX)

Table 1

		FEMALES (n=634)						MALES (n=487)					
VARIABLE	**UNIT**	**MIN**	**MAX**	**RANGE**	**MEDIAN**	**MEAN**	**SD**	**MIN**	**MAX**	**RANGE**	**MEDIAN**	**MEAN**	**SD**
Age	years	14.6	16.8	2.2	15.3	15.4	0.2	14.5	17.0	2.5	15.3	15.4	0.2
Height	cm	148.6	183.3	34.7	164.7	164.9	5.9	145.0	195.0	50.0	174.8	174.4	7.6
Weight	kg	38.4	93.2	54.8	56.7	58.3	9.2	32.3	102.4	70.1	62.0	62.7	10.0
F-MASS	kg	5.7	46.1	40.4	17.0	18.0	7.0	2.2	46.7	44.4	8.1	10.0	6.4
L-MASS	kg	28.4	49.3	21.0	36.8	37.2	3.8	26.3	74.0	47.7	50.1	49.9	6.5
BMD_C	mg/cm^3	1041.6	1188.4	146.8	1125.9	1125.0	21.8	958.1	1154.0	195.9	1076.5	1074.5	33.4
BMC_C	mg	190.5	430.9	240.5	307.7	309.7	40.5	161.9	505.9	344.1	353.6	354.9	50.6
BA_C	mm	169.6	379.7	210.1	273.9	275.4	36.0	162.0	460.7	298.7	330.3	330.2	44.8
CSA	mm	239.8	614.8	374.9	378.6	385.7	54.2	281.8	680.2	398.5	463.0	467.5	63.1
PC	mm	54.9	87.9	33.0	69.0	69.5	4.8	59.5	92.5	33.0	76.3	76.5	5.2
EC	mm	24.0	60.7	36.6	36.3	36.9	5.1	26.4	60.8	34.4	40.9	41.2	5.6
CT	mm	3.4	7.0	3.6	5.2	5.2	0.6	3.3	7.3	4.0	5.6	5.6	0.7
SSI	cm^3	450.7	1543.3	1092.6	899.5	921.4	178.2	455.1	1903.1	1447.9	1158.1	1168.8	226.5
Citrate*	mmol/L	0.05	0.18	0.13	0.11	0.11	0.02	0.06	0.19	0.13	0.12	0.12	0.02
β-CTX	ng/ml	0.30	2.29	1.99	0.69	0.74	0.25	0.44	3.26	2.82	1.42	1.49	0.51

Table showing characteristics of participants included in the analysis of metabolic and tibial pQCT-derived parameters, as minimum, maximum, range, median, mean, standard deviation (SD). n = Sample size; AGE = age of subject at attendance of the teen focus three clinic (TF3); F-MASS = total body fat mass; L-MASS = total body lean mass; BMD_C = cortical bone mineral density; BMC_C = cortical bone mineral content; BA_c = cortical bone area; CSA = cross-sectional area; PC = periosteal circumference; EC = endosteal circumference; CT = cortical thickness, SSI = strength strain index, and β-CTX = β-C-telopeptides of type I collagen. *Normal reference range for serum/plasma citrate in adults ranges from 1.7 - 3.0 mg/dL, equating to 0.09 – 0.16mmol/L. Descriptive statistics for the combined sample of males and females and corresponding z-scores (parenthesis) expressed relative to the entire Teen Focus 3 cohort of 5,171 subjects are as follows: Height: [min = 145.0 cm (−2.89 sd), max = 195.0 (3.07), median = 168.3 (−0.112), mean = 169.0 (−0.025) and standard deviation = 8.186

(0.976)] and weight [min = 32.3 kg (−2.47 sd), max = 102.4 (3.47), median = 59.5 (−0.166), mean = 60.21 (−0.106) and sd = 9.80 (0.830)]. Breakdown of participants according to Tanner stage and sex (female/male) at age 13.5 years: Stage 1 (n=31/54); Stage 2 (n=70/118); Stage 3 (n=154/133); Stage 4 (n=250/151) and Stage 5 (n=129/31).

Table 2

STANDARDISED OUTCOME	MODEL 1				MODEL 2				MODEL 3				MODEL 4			
	β	**CI95L**	**CI95U**	***P***	***β***	**CI95L**	**CI95U**	***P***	***β***	**CI95L**	**CI95U**	***P***	***β***	**CI95L**	**CI95U**	***P***
BMDC	-0.21	-0.25	-0.17	1.3×10^{-21}	-0.13	-0.18	-0.09	1.9×10^{-10}	-0.14	-0.18	-0.09	1.4×10^{-10}	-0.14	-0.18	-0.09	2.42×10^{-10}
BMCC	-0.13	-0.18	-0.07	3.8×10^{-6}	-0.07	-0.12	-0.01	0.02	-0.08	-0.12	-0.03	1.5×10^{-3}	0.02	-0.02	0.06	0.41
BAC	-0.08	-0.13	-0.03	2.5×10^{-3}	-0.03	-0.09	0.02	0.18	-0.04	-0.09	0.00	0.05	0.04	0.01	0.08	0.02
CSA	-0.03	-0.08	0.02	0.25	0.00	-0.05	0.05	0.99	-0.01	-0.05	0.03	0.65	0.06	0.03	0.10	1.2×10^{-3}
PC	-0.03	-0.08	0.02	0.25	0.00	-0.05	0.05	0.99	-0.01	-0.05	0.03	0.64	0.06	0.03	0.10	1.2×10^{-3}
EC	0.05	-0.01	0.10	0.10	0.05	-0.01	0.10	0.12	0.04	-0.02	0.10	0.16	0.07	0.01	0.13	0.01
CT	-0.11	-0.17	-0.06	1.0×10^{-4}	-0.07	-0.12	-0.01	0.02	-0.07	-0.13	-0.02	0.01	0.00	-0.05	0.05	0.94
SSI	-0.08	-0.13	-0.03	3.1×10^{-3}	-0.03	-0.08	0.02	0.25	-0.04	-0.09	0.00	0.06	0.04	0.01	0.08	0.02
β-CTX*	0.28	0.24	0.31	7.9×10^{-48}	0.22	0.18	0.25	1.6×10^{-32}	0.22	0.18	0.25	1.48×10^{-32}	0.20	0.16	0.23	5.0×10^{-27}

Table shows regression analyses of citrate versus pQCT variables and β-CTX in 1121 participants aged 15.4 (634 females, 487 males). Outcome and exposure measures were standardised prior to analysis (mean = 0 and standard deviation = 1). Model 1 = adjustment for age and sex. Model 2 = model 1 in addition to Tanner stage, Model 3 = model 2 in addition to height, and model 4 = model 3 in addition to lean mass and fat mass. *β* = SD change in outcome per SD increase in citrate; CI95L = lower 95% confidence estimate of *β*; CI95U = upper 95% confidence estimate of *β*; *P* = strength of evidence against the null hypothesis of no association between the outcome and exposure variable; BMDC = cortical bone mineral density; BMCC = cortical bone mineral content; BAC = cortical bone area; CSA = cross sectional area; PC = periosteal circumference; EC = endosteal circumference; CT = cortical thickness; SSI = strength strain index and β-CTX* = β-C-telopeptides of type I collagen (log transformed to normality) and standardized to z-scores prior to regression analysis. Note: All regression models involving β-CTX were adjusted for time of clinic attendance in addition to model specific covariates.

SUPPLEMENTARY DATA:

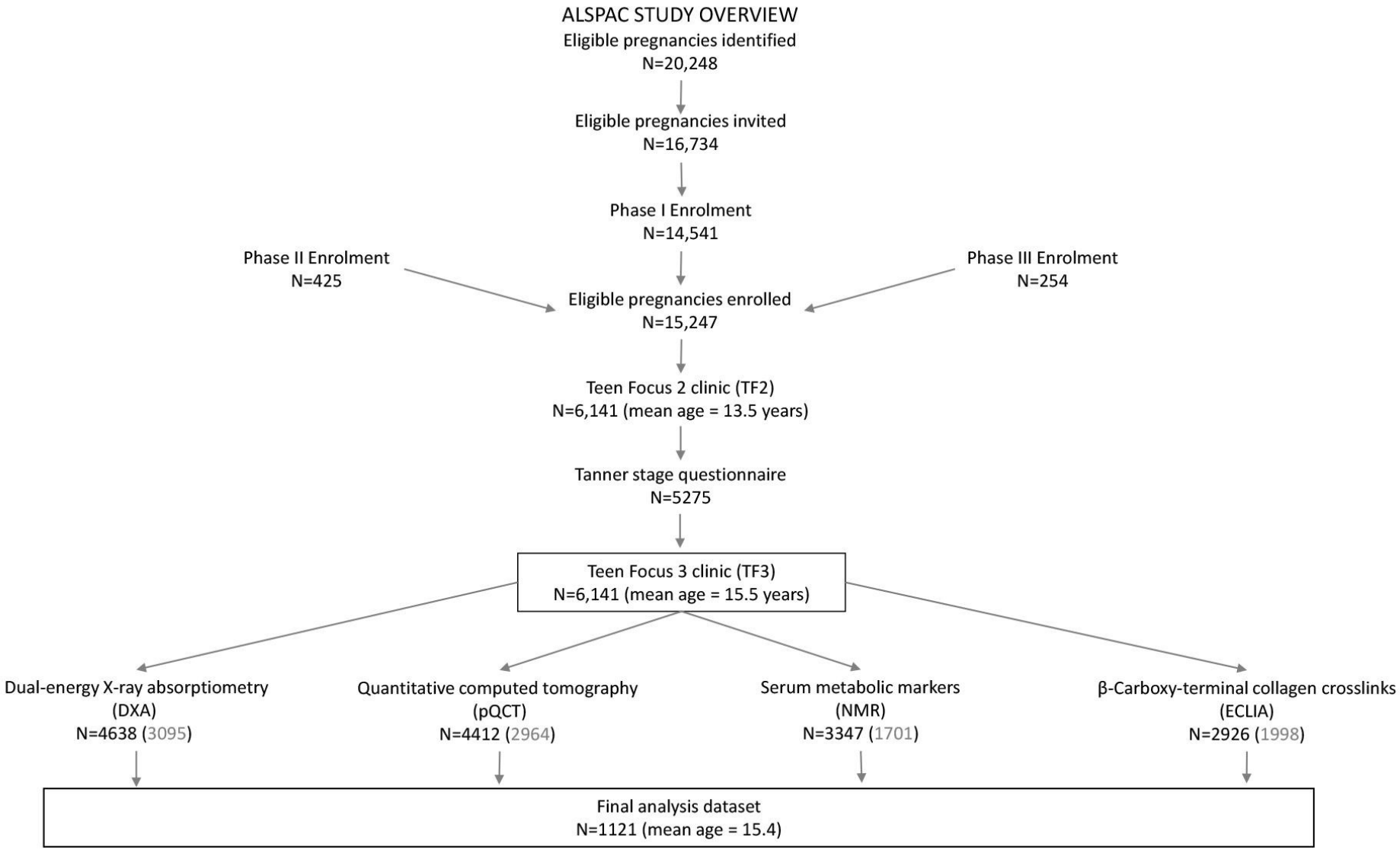

Figure S1. Flow diagram illustrating ALSPAC subject enrollment and the source of the data used in the present study. ALSPAC is a longitudinal population-based birth cohort that recruited 14,541 pregnant women resident in Avon, UK with expected dates of delivery 1st April 1991 to 31st December 1992. 14,541 is the initial number of pregnancies for which the mother enrolled in the ALSPAC study and had either returned at least one questionnaire or attended a "Children in Focus" clinic by 19/07/99. Of these initial pregnancies, there was a total of 14,676 foetuses, resulting in 14,062 live births and 13,988 children who were alive at 1 year of age. When the oldest children were approximately 7 years of age, an attempt was made to bolster the initial sample with eligible cases who had failed to join the study originally. As a result, when considering variables collected from the age of seven onwards (and potentially abstracted from obstetric notes) there are data available for more than the 14,541 pregnancies mentioned above. The number of new pregnancies not in the initial sample (known as Phase I enrolment) that are currently represented on the built files and reflecting enrolment status at the age of 18 is 706 (452 and 254 recruited during Phases II and III respectively), resulting in an additional 713 children being enrolled. The phases of enrolment are described in more detail on the webstite (http://www.alspac.bris.ac.uk) and in the cohort profile paper [7,8]. The total sample size for analyses using any data collected after the age of seven is therefore 15,247 pregnancies, resulting in 15,458 foetuses. Of this total sample of 15,458 foetuses, 14,775 were live births and 14,701 were alive at 1 year of age. A 10% sample of the ALSPAC cohort, known as the Children in Focus (CiF) group, attended clinics at the University of Bristol at various time intervals between 4 to 61 months of age. The CiF group were chosen at random from the last 6 months of ALSPAC births (1432 families attended at least one clinic). Excluded were those mothers who had moved out of the area or were lost to follow-up, and those partaking in another study of infant development in Avon. The present study is based on research clinics to which the whole cohort was invited and held when participants were a mean age of 13.5 years (Teen Focus
2) for the Tanner stage questionnaire (N=5275 valid questionnaires) and 15.5 years for imaging and serum metabolic measures (Teen Focus 3). Numbers coloured in black correspond to numbers of subjects with valid imaging / serum measures and grey coloured represent number of subjects with imaging / serum and Tanner stage questionnaire data.

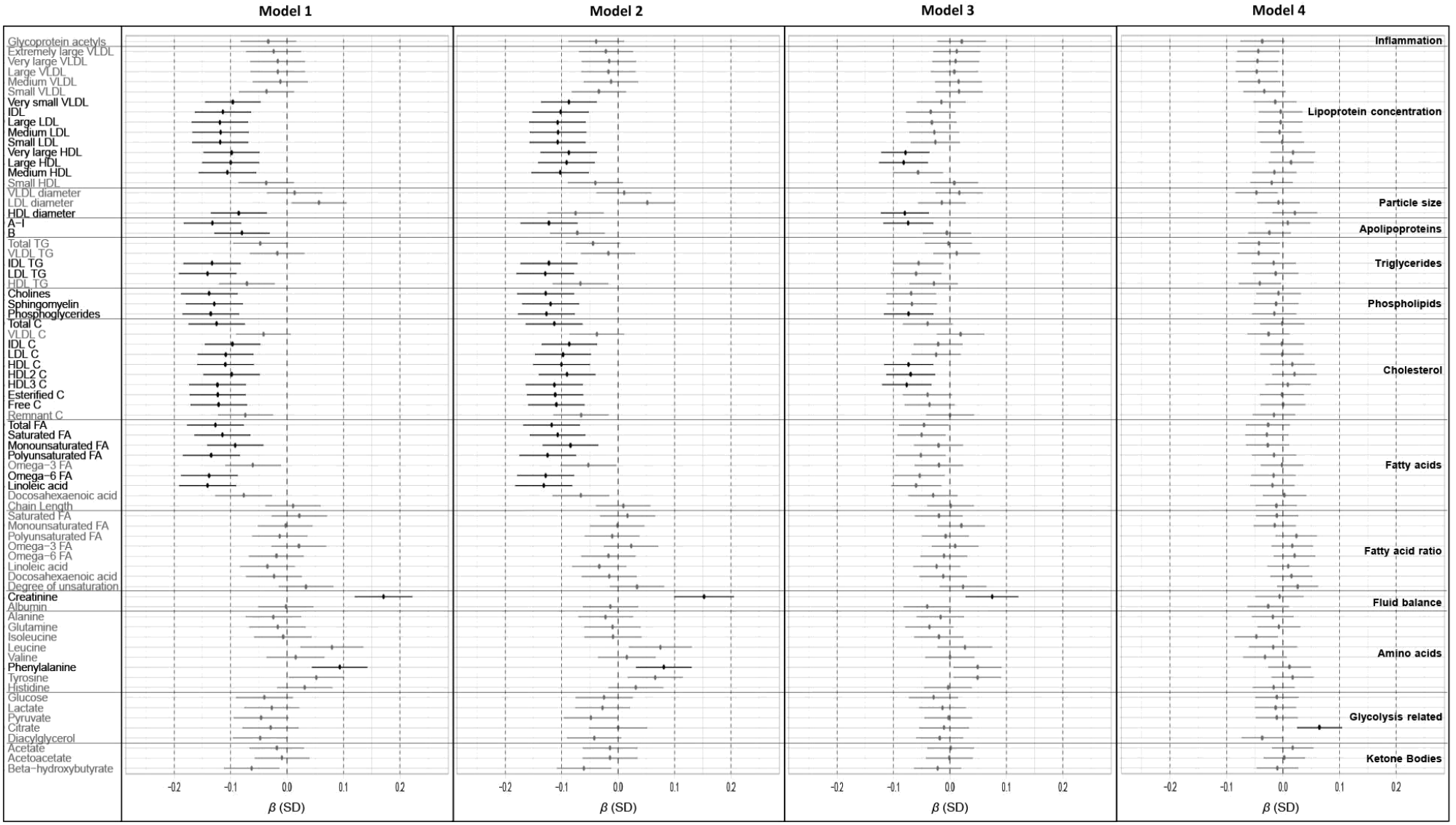
Model 1
Model 2
Model 3
Model 4
Glycoprotein acetyls
Extremely large VLDL
Very large VLDL
Large VLDL
Medium VLDL
Small VLDL
Very small VLDL
IDL
Large LDL
Medium LDL
Small LDL
Very large HDL
Large HDL
Medium HDL
Small HDL
VLDL diameter
LDL diameter
HDL diameter
A−I
B
Total TG
VLDL TG
IDL TG
LDL TG
HDL TG
Cholines
Sphingomyelin
Phosphoglycerides
Total C
VLDL C
IDL C
LDL C
HDL C
HDL2 C
HDL3 C
Esterified C
Free C
Remnant C
Total FA
Saturated FA
Monounsaturated FA
Polyunsaturated FA
Omega−3 FA
Omega−6 FA
Linoleic acid
Docosahexaenoic acid
Chain Length
Saturated FA
Monounsaturated FA
Polyunsaturated FA
Omega−3 FA
Omega−6 FA
Linoleic acid
Docosahexaenoic acid
Degree of unsaturation
Creatinine
Albumin
Alanine
Glutamine
Isoleucine
Leucine
Valine
Phenylalanine
Tyrosine
Histidine
Glucose
Lactate
Pyruvate
Citrate
Diacylglycerol
Acetate
Acetoacetate
Beta−hydroxybutyrate
Inflammation
Lipoprotein concentration
Particle size
Apolipoproteins
Triglycerides
Phospholipids
Cholesterol
Fatty acids
Fatty acid ratio
Fluid balance
Amino acids
Glycolysis related
Ketone Bodies
−0.2
−0.1
0.0
0.1
0.2
β (SD)

Figure S2: Summary of relationships between 73 representative serum metabolites and pQCT derived periosteal circumference measured at the tibia.

Outcome and exposure measures were standardised prior to analysis (mean = 0 and standard deviation = 1). Periosteal circumference [PC (outcome)] was regressed on 73 representative metabolites (exposure) correcting for age and sex (model 1). Stepwise regression involved incorporating additional covariates, namely Tanner stage (model 2), height (model 3), and lean mass, fat mass (model 4). A Bonferroni multiple testing threshold of $P < 0.002$ (0.05 / 22 principal components) was used to identify metabolites that were robustly associated with PC. Note: Point estimates of β are expressed as SD change in outcome per SD change in metabolite and denoted by circles with corresponding 95% CIs denoted by bars. Observations with sufficient strength of evidence to reject the null hypothesis of no association between the metabolite and PC are coloured in black and their corresponding names highlighted in black. Observations not meeting the significance threshold are coloured in grey. Metabolites are classified into 13 categories, namely: Inflammation, Lipoprotein concentration, Particle size, Apolipoproteins, Triglycerides, Phospholipids, Cholesterol, Fatty acids, Fatty acid ratios, Fluid balance, Amino acids, Glycolosis related and Ketone bodies. VLDL = very low density lipoprotein, LDL = low density lipoprotein, IDL = intermediate density lipoprotein, HDL = high density lipoprotein, TG = triglycerides, C = cholesterol and FA = Fatty acids

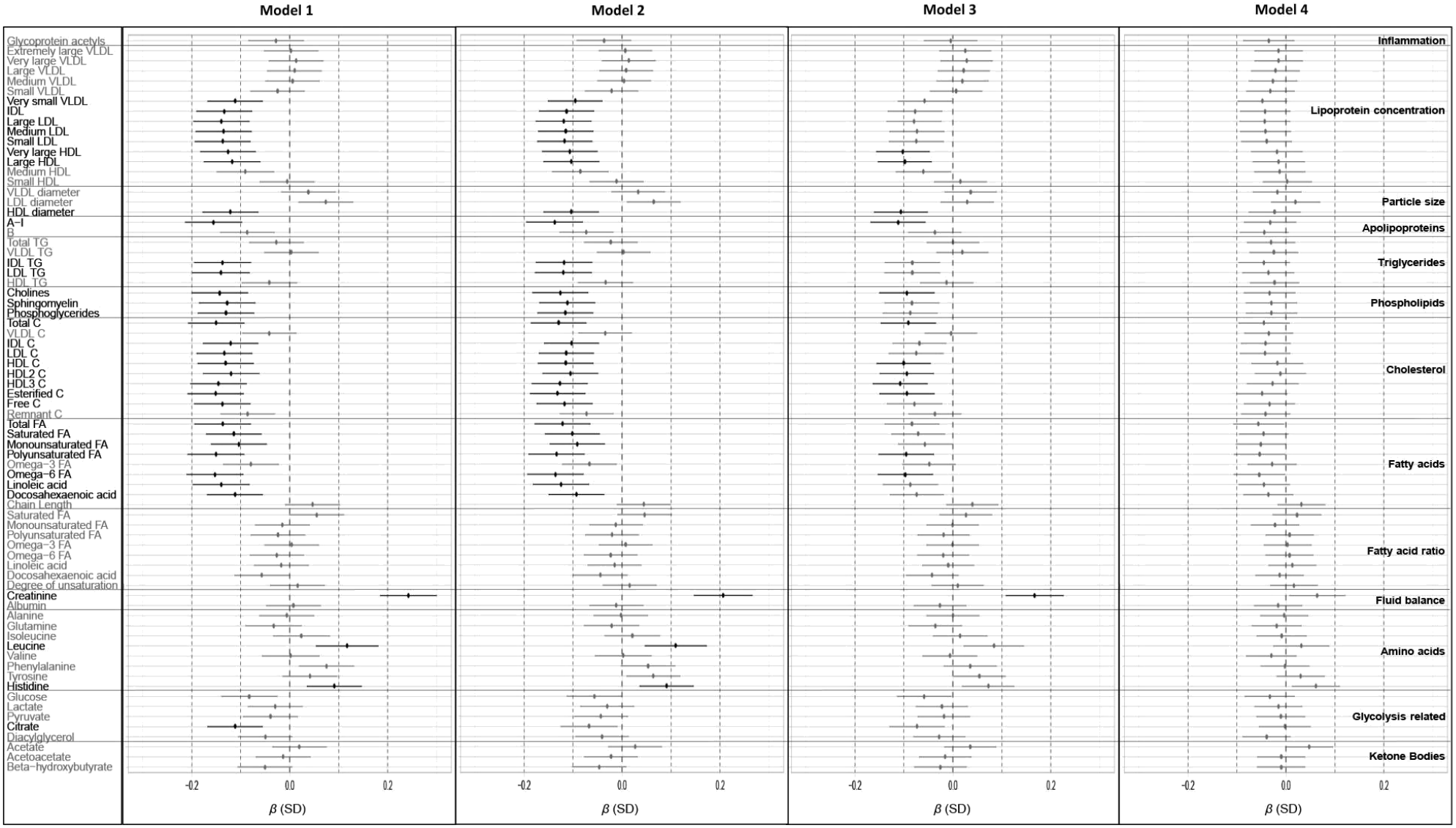
Model 1
Model 2
Model 3
Model 4
Glycoprotein acetyls
Extremely large VLDL
Very large VLDL
Large VLDL
Medium VLDL
Small VLDL
Very small VLDL
IDL
Large LDL
Medium LDL
Small LDL
Very large HDL
Large HDL
Medium HDL
Small HDL
VLDL diameter
LDL diameter
HDL diameter
A−I
B
Total TG
VLDL TG
IDL TG
LDL TG
HDL TG
Cholines
Sphingomyelin
Phosphoglycerides
Total C
VLDL C
IDL C
LDL C
HDL C
HDL2 C
HDL3 C
Esterified C
Free C
Remnant C
Total FA
Saturated FA
Monounsaturated FA
Polyunsaturated FA
Omega−3 FA
Omega−6 FA
Linoleic acid
Docosahexaenoic acid
Chain Length
Saturated FA
Monounsaturated FA
Polyunsaturated FA
Omega−3 FA
Omega−6 FA
Linoleic acid
Docosahexaenoic acid
Degree of unsaturation
Creatinine
Albumin
Alanine
Glutamine
Isoleucine
Leucine
Valine
Phenylalanine
Tyrosine
Histidine
Glucose
Lactate
Pyruvate
Citrate
Diacylglycerol
Acetate
Acetoacetate
Beta−hydroxybutyrate
Inflammation
Lipoprotein concentration
Particle size
Apolipoproteins
Triglycerides
Phospholipids
Cholesterol
Fatty acids
Fatty acid ratio
Fluid balance
Amino acids
Glycolysis related
Ketone Bodies
-0.2
0.0
0.2
β (SD)

Figure S3: Summary of relationships between 73 representative serum metabolites and pQCT derived cortical thickness measured at the tibia.

Outcome and exposure measures were standardised prior to analysis (mean = 0 and standard deviation = 1). Cortical thickness [CT (outcome)] was regressed on 73 representative metabolites (exposure) correcting for age and sex (model 1). Stepwise regression involved incorporating additional covariates, namely Tanner stage (model 2), height (model 3), and lean mass and fat mass (model 4). A Bonferroni multiple testing threshold of $P < 0.002$ (0.05 / 22 principal components) was used to identify metabolites that were robustly associated with PC. Note: Point estimates of β are expressed as SD change in outcome per SD change in metabolite and denoted by circles with corresponding 95% CIs denoted by bars. Observations with sufficient strength of evidence to reject the null hypothesis of no association between the metabolite and CT are coloured in black and their corresponding names highlighted in black. Observations not meeting the significance threshold are coloured in grey. Metabolites are classified into 13 categories, namely: Inflammation, Lipoprotein concentration, Particle size, Apolipoproteins, Triglycerides, Phospholipids, Cholesterol, Fatty acids, Fatty acid ratios, Fluid balance, Amino acids, Glycolosis related and Ketone bodies. VLDL = very low density lipoprotein, LDL = low density lipoprotein, IDL = intermediate density lipoprotein, HDL = high density lipoprotein, TG = triglycerides, C = cholesterol and FA = Fatty acids.

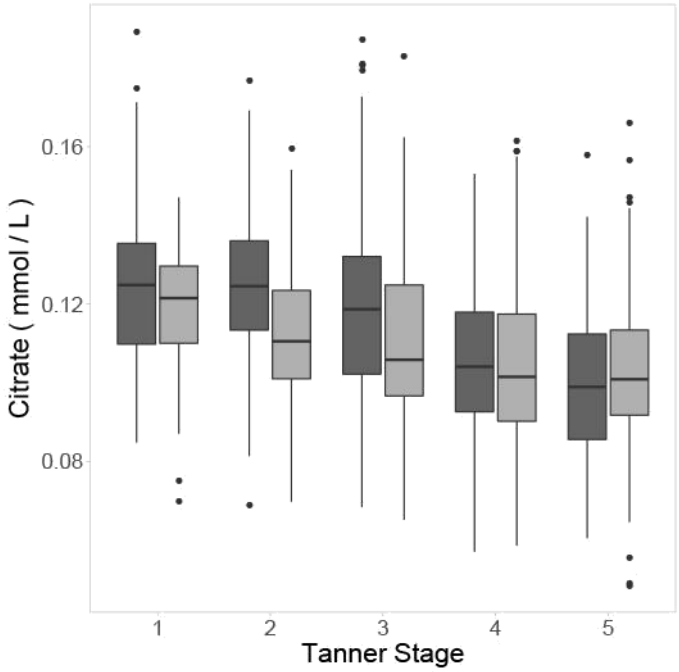

Figure S4. Box and whisker plot describing the relationship between Tanner stage at age 13.5 (x-axis) and serum citrate at age 15.5 (y-axis) stratified by sex (males = dark grey, females = light grey).

Table S1.

STANDARDISED OUTCOME = BMDc	MODEL 1						MODEL 2						MODEL 3						MODEL 4					
STANDARDISED EXPOSURE	***β****	***CI95L***	***CI95U***	***P***	**R^2**	**AIC**	***β****	***CI95L***	***CI95U***	***P***	***R2***	***AIC***	***β****	***CI95L***	***CI95U***	***P***	***R2***	***AIC***	***β****	***CI95L***	***CI95U***	***P***	***R2***	***AIC***
Glycoprotein acetyls[1]	0.01	-0.03	0.06	5.80E-01	0.46	-693	0	-0.04	0.04	8.65E-01	0.56	-901	0.00	-0.04	0.04	9.55E-01	0.56	-901	0.01	-0.03	0.05	7.15E-01	0.56	-904
Extremely large VLDL[2]	-0.03	-0.07	0.01	1.46E-01	0.47	-695	-0.02	-0.06	0.01	2.13E-01	0.56	-902	-0.02	-0.06	0.02	2.63E-01	0.56	-902	-0.02	-0.06	0.02	2.80E-01	0.56	-905
Very large VLDL[2]	-0.02	-0.06	0.02	3.50E-01	0.46	-694	-0.02	-0.06	0.02	3.37E-01	0.56	-901	-0.02	-0.06	0.02	3.90E-01	0.56	-902	-0.02	-0.06	0.02	3.91E-01	0.56	-904
Large VLDL[2]	-0.01	-0.06	0.03	5.09E-01	0.46	-693	-0.02	-0.06	0.02	4.09E-01	0.56	-901	-0.01	-0.05	0.02	4.65E-01	0.56	-902	-0.02	-0.06	0.02	4.54E-01	0.56	-904
Medium VLDL[2]	-0.02	-0.06	0.02	3.76E-01	0.46	-694	-0.02	-0.06	0.02	2.80E-01	0.56	-902	-0.02	-0.06	0.02	3.30E-01	0.56	-902	-0.02	-0.06	0.02	3.17E-01	0.56	-905
Small VLDL[2]	-0.04	-0.09	0	4.86E-02	0.47	-697	-0.04	-0.08	0	6.53E-02	0.56	-904	-0.03	-0.07	0.01	9.69E-02	0.56	-904	-0.03	-0.07	0.01	9.79E-02	0.56	-906
Very small VLDL[2]	**-0.09**	**-0.14**	**-0.05**	**3.37E-05**	**0.47**	**-710**	**-0.06**	**-0.1**	**-0.02**	**1.64E-03**	**0.56**	**-910**	-0.06	-0.10	-0.02	3.52E-03	0.56	-910	-0.06	-0.10	-0.02	5.43E-03	0.56	-911
IDL[2]	**-0.1**	**-0.15**	**-0.06**	**3.71E-06**	**0.47**	**-714**	**-0.07**	**-0.11**	**-0.03**	**1.22E-03**	**0.56**	**-911**	-0.06	-0.10	-0.02	2.57E-03	0.56	-910	-0.06	-0.10	-0.02	4.60E-03	0.56	-912
Large LDL[2]	**-0.11**	**-0.15**	**-0.06**	**3.47E-06**	**0.47**	**-714**	**-0.07**	**-0.11**	**-0.03**	**1.19E-03**	**0.56**	**-911**	-0.06	-0.11	-0.02	2.66E-03	0.56	-910	-0.06	-0.10	-0.02	5.60E-03	0.56	-911
Medium LDL[2]	**-0.1**	**-0.15**	**-0.06**	**5.64E-06**	**0.47**	**-713**	**-0.07**	**-0.11**	**-0.03**	**1.48E-03**	**0.56**	**-911**	-0.06	-0.10	-0.02	3.37E-03	0.56	-910	-0.06	-0.10	-0.02	7.16E-03	0.56	-911
Small LDL[2]	**-0.1**	**-0.15**	**-0.06**	**3.68E-06**	**0.47**	**-714**	**-0.07**	**-0.11**	**-0.03**	**1.09E-03**	**0.56**	**-911**	-0.06	-0.10	-0.02	2.58E-03	0.56	-910	-0.06	-0.10	-0.02	5.77E-03	0.56	-911
Very large HDL[2]	**-0.08**	**-0.12**	**-0.03**	**8.54E-04**	**0.47**	**-704**	-0.04	-0.08	0	5.57E-02	0.56	-904	-0.04	-0.08	0.00	5.96E-02	0.56	-905	-0.04	-0.08	0.00	7.06E-02	0.56	-907
Large HDL[2]	-0.05	-0.1	-0.01	1.65E-02	0.47	-699	-0.03	-0.07	0.01	1.59E-01	0.56	-903	-0.03	-0.07	0.01	1.70E-01	0.56	-903	-0.03	-0.07	0.01	1.97E-01	0.56	-905
Medium HDL[2]	-0.03	-0.08	0.01	1.35E-01	0.47	-695	-0.03	-0.07	0.02	2.25E-01	0.56	-902	-0.02	-0.06	0.02	2.94E-01	0.56	-902	-0.02	-0.06	0.02	4.16E-01	0.56	-904
Small HDL[2]	0.01	-0.03	0.05	6.48E-01	0.46	-693	0	-0.04	0.04	9.90E-01	0.56	-901	0.00	-0.04	0.04	8.63E-01	0.56	-901	0.01	-0.03	0.05	7.24E-01	0.56	-904
VLDL diameter[3]	0.02	-0.03	0.06	4.51E-01	0.46	-693	0.01	-0.03	0.05	7.19E-01	0.56	-901	0.01	-0.03	0.05	7.01E-01	0.56	-901	0.01	-0.03	0.05	7.49E-01	0.56	-904
LDL diameter[3]	0.06	0.01	0.1	1.06E-02	0.47	-699	0.04	0	0.08	4.37E-02	0.56	-905	0.04	0.00	0.08	7.40E-02	0.56	-904	0.03	-0.01	0.07	1.27E-01	0.56	-906
HDL diameter[3]	-0.06	-0.11	-0.02	5.88E-03	0.47	-700	-0.03	-0.07	0.01	1.41E-01	0.56	-903	-0.03	-0.07	0.01	1.36E-01	0.56	-903	-0.03	-0.07	0.01	1.44E-01	0.56	-906
Apolopoprotein A-I[4]	**-0.09**	**-0.13**	**-0.04**	**1.25E-04**	**0.47**	**-708**	-0.06	-0.1	-0.01	8.85E-03	0.56	-907	-0.05	-0.09	-0.01	1.41E-02	0.56	-907	-0.05	-0.09	-0.01	2.50E-02	0.56	-909
Apolopoprotein B[4]	**-0.08**	**-0.13**	**-0.04**	**1.85E-04**	**0.47**	**-707**	-0.06	-0.1	-0.02	3.66E-03	0.56	-909	-0.05	-0.09	-0.01	7.30E-03	0.56	-908	-0.05	-0.09	-0.01	1.06E-02	0.56	-910
Total TG[5]	-0.04	-0.08	0	7.29E-02	0.47	-696	-0.03	-0.07	0.01	1.14E-01	0.56	-903	-0.03	-0.07	0.01	1.53E-01	0.56	-903	-0.03	-0.07	0.01	1.56E-01	0.56	-906
VLDL TG[5]	-0.02	-0.06	0.02	3.57E-01	0.46	-694	-0.02	-0.06	0.02	2.94E-01	0.56	-902	-0.02	-0.06	0.02	3.48E-01	0.56	-902	-0.02	-0.06	0.02	3.37E-01	0.56	-905
IDL TG[5]	**-0.09**	**-0.14**	**-0.05**	**6.89E-05**	**0.47**	**-709**	-0.06	-0.1	-0.02	6.92E-03	0.56	-908	-0.05	-0.10	-0.01	1.31E-02	0.56	-907	-0.05	-0.09	-0.01	1.71E-02	0.56	-909
LDL TG[5]	**-0.1**	**-0.14**	**-0.05**	**3.83E-05**	**0.47**	**-710**	-0.06	-0.1	-0.02	7.34E-03	0.56	-908	-0.05	-0.10	-0.01	1.41E-02	0.56	-907	-0.05	-0.09	-0.01	2.06E-02	0.56	-909
HDL TG[5]	-0.05	-0.09	0	3.29E-02	0.47	-697	-0.03	-0.07	0.01	9.39E-02	0.56	-903	-0.03	-0.07	0.01	1.24E-01	0.56	-904	-0.03	-0.07	0.01	1.20E-01	0.56	-906
Cholines[6]	**-0.1**	**-0.14**	**-0.05**	**3.53E-05**	**0.47**	**-710**	-0.06	-0.11	-0.02	2.28E-03	0.56	-910	-0.06	-0.10	-0.02	4.30E-03	0.56	-909	-0.06	-0.10	-0.02	7.46E-03	0.56	-911

Sphingomyelin[6]	**-0.09**	**-0.14**	**-0.05**	**7.52E-05**	**0.47**	**-708**	-0.06	-0.1	-0.02	3.15E-03	0.56	-909	-0.06	-0.10	-0.02	5.49E-03	0.56	-909	-0.06	-0.10	-0.01	8.30E-03	0.56	-911
Phosphoglycerides[6]	**-0.09**	**-0.13**	**-0.04**	**2.01E-04**	**0.47**	**-707**	-0.06	-0.1	-0.02	4.60E-03	0.56	-909	-0.06	-0.10	-0.01	7.98E-03	0.56	-908	-0.05	-0.10	-0.01	1.28E-02	0.56	-910
Total C[7]	**-0.11**	**-0.15**	**-0.07**	**1.42E-06**	**0.48**	**-716**	**-0.07**	**-0.11**	**-0.03**	**6.02E-04**	**0.56**	**-912**	**-0.07**	**-0.11**	**-0.03**	**1.37E-03**	**0.56**	**-911**	-0.06	-0.10	-0.02	3.19E-03	0.56	-912
VLDL C[7]	-0.06	-0.1	-0.02	7.21E-03	0.47	-700	-0.05	-0.09	-0.01	2.15E-02	0.56	-906	-0.04	-0.08	0.00	3.56E-02	0.56	-906	-0.04	-0.08	0.00	4.07E-02	0.56	-908
IDL C[7]	**-0.1**	**-0.14**	**-0.05**	**2.04E-05**	**0.47**	**-711**	-0.06	-0.1	-0.02	2.07E-03	0.56	-910	-0.06	-0.10	-0.02	4.18E-03	0.56	-909	-0.06	-0.10	-0.01	7.77E-03	0.56	-911
LDL C[7]	**-0.1**	**-0.15**	**-0.06**	**6.85E-06**	**0.47**	**-713**	-0.07	-0.11	-0.03	1.50E-03	0.56	-911	-0.06	-0.10	-0.02	3.29E-03	0.56	-910	-0.06	-0.10	-0.02	6.86E-03	0.56	-911
HDL C[7]	-0.07	-0.11	-0.02	3.07E-03	0.47	-702	-0.04	-0.08	0	6.25E-02	0.56	-904	-0.04	-0.08	0.00	7.73E-02	0.56	-904	-0.03	-0.08	0.01	1.12E-01	0.56	-906
HDL2 C[7]	-0.05	-0.1	-0.01	1.58E-02	0.47	-699	-0.03	-0.07	0.01	1.53E-01	0.56	-903	-0.03	-0.07	0.01	1.76E-01	0.56	-903	-0.03	-0.07	0.02	2.41E-01	0.56	-905
HDL3 C[7]	**-0.09**	**-0.13**	**-0.04**	**1.39E-04**	**0.47**	**-707**	-0.05	-0.1	-0.01	1.07E-02	0.56	-907	-0.05	-0.09	-0.01	1.52E-02	0.56	-907	-0.05	-0.09	-0.01	2.42E-02	0.56	-909
Esterified C[7]	**-0.11**	**-0.15**	**-0.06**	**2.20E-06**	**0.47**	**-715**	**-0.07**	**-0.11**	**-0.03**	**7.09E-04**	**0.56**	**-912**	**-0.07**	**-0.11**	**-0.03**	**1.58E-03**	**0.56**	**-911**	-0.06	-0.10	-0.02	3.52E-03	0.56	-912
Free C[7]	**-0.11**	**-0.15**	**-0.06**	**2.07E-06**	**0.48**	**-715**	**-0.07**	**-0.11**	**-0.03**	**8.55E-04**	**0.56**	**-912**	**-0.07**	**-0.11**	**-0.02**	**1.91E-03**	**0.56**	**-911**	-0.06	-0.10	-0.02	4.50E-03	0.56	-912
Remnant C[7]	**-0.08**	**-0.13**	**-0.04**	**1.73E-04**	**0.47**	**-707**	-0.06	-0.1	-0.02	3.49E-03	0.56	-909	-0.06	-0.10	-0.02	6.93E-03	0.56	-909	-0.05	-0.09	-0.01	1.06E-02	0.56	-910
Total FA[8]	**-0.09**	**-0.14**	**-0.05**	**6.34E-05**	**0.47**	**-709**	-0.06	-0.1	-0.02	2.59E-03	0.56	-910	-0.06	-0.10	-0.02	5.42E-03	0.56	-909	-0.05	-0.10	-0.01	9.88E-03	0.56	-910
Saturated FA[8]	**-0.07**	**-0.12**	**-0.03**	**9.89E-04**	**0.47**	**-704**	-0.05	-0.09	-0.01	1.26E-02	0.56	-907	-0.05	-0.09	-0.01	2.15E-02	0.56	-907	-0.04	-0.09	0.00	3.06E-02	0.56	-908
Monounsaturated FA[8]	**-0.09**	**-0.13**	**-0.04**	**8.93E-05**	**0.47**	**-708**	**-0.07**	**-0.11**	**-0.03**	**1.38E-03**	**0.56**	**-911**	-0.06	-0.10	-0.02	2.79E-03	0.56	-910	-0.06	-0.10	-0.02	5.10E-03	0.56	-912
Polyunsaturated FA[8]	**-0.08**	**-0.13**	**-0.04**	**2.43E-04**	**0.47**	**-706**	-0.05	-0.09	-0.01	1.19E-02	0.56	-907	-0.05	-0.09	-0.01	2.30E-02	0.56	-906	-0.04	-0.09	0.00	3.92E-02	0.56	-908
Omega-3 FA[8]	-0.05	-0.1	-0.01	1.68E-02	0.47	-698	-0.03	-0.07	0.01	1.42E-01	0.56	-903	-0.03	-0.07	0.01	1.78E-01	0.56	-903	-0.03	-0.07	0.01	2.11E-01	0.56	-905
Omega-6 FA[8]	**-0.08**	**-0.13**	**-0.04**	**2.55E-04**	**0.47**	**-706**	-0.05	-0.09	-0.01	1.14E-02	0.56	-907	-0.05	-0.09	-0.01	2.23E-02	0.56	-906	-0.04	-0.09	0.00	3.87E-02	0.56	-908
Linoleic acid[8]	**-0.08**	**-0.12**	**-0.03**	**9.70E-04**	**0.47**	**-704**	-0.05	-0.09	-0.01	2.38E-02	0.56	-906	-0.04	-0.08	0.00	4.37E-02	0.56	-905	-0.04	-0.08	0.00	6.13E-02	0.56	-907
Docosahexaenoic acid[8]	**-0.08**	**-0.12**	**-0.03**	**7.77E-04**	**0.47**	**-704**	-0.04	-0.08	0	4.19E-02	0.56	-905	-0.04	-0.08	0.00	5.65E-02	0.56	-905	-0.04	-0.08	0.01	8.94E-02	0.56	-907
Chain Length[8]	-0.01	-0.05	0.04	7.84E-01	0.46	-693	-0.01	-0.05	0.03	6.19E-01	0.56	-901	-0.01	-0.05	0.03	5.97E-01	0.56	-901	-0.01	-0.05	0.03	6.25E-01	0.56	-904
Saturated FA %[9]	0.05	0	0.09	3.83E-02	0.47	-697	0.03	-0.01	0.07	1.29E-01	0.56	-903	0.03	-0.01	0.07	1.66E-01	0.56	-903	0.02	-0.02	0.06	2.38E-01	0.56	-905
Monounsaturated FA %[9]	-0.04	-0.09	0	4.30E-02	0.47	-697	-0.04	-0.08	0	4.51E-02	0.56	-905	-0.04	-0.08	0.00	5.44E-02	0.56	-905	-0.04	-0.08	0.00	6.74E-02	0.56	-907
Polyunsaturated FA %[9]	0.02	-0.03	0.06	4.93E-01	0.46	-693	0.02	-0.02	0.06	2.82E-01	0.56	-902	0.02	-0.02	0.06	2.77E-01	0.56	-902	0.02	-0.02	0.06	2.62E-01	0.56	-905
Omega-3 FA %[9]	0	-0.04	0.05	8.77E-01	0.46	-693	0.01	-0.03	0.05	6.50E-01	0.56	-901	0.01	-0.03	0.05	6.89E-01	0.56	-901	0.01	-0.03	0.05	7.11E-01	0.56	-904
Omega-6 FA %[9]	0.01	-0.03	0.06	4.96E-01	0.46	-693	0.02	-0.02	0.06	3.12E-01	0.56	-902	0.02	-0.02	0.06	2.99E-01	0.56	-902	0.02	-0.02	0.06	2.80E-01	0.56	-905
Linoleic acid %[9]	0.01	-0.03	0.06	5.14E-01	0.46	-693	0.02	-0.02	0.06	3.72E-01	0.56	-901	0.02	-0.02	0.06	3.51E-01	0.56	-902	0.02	-0.02	0.06	3.83E-01	0.56	-904
Docosahexaenoic acid %[9]	-0.04	-0.08	0.01	9.08E-02	0.47	-696	-0.02	-0.05	0.02	4.55E-01	0.56	-901	-0.01	-0.05	0.02	4.63E-01	0.56	-902	-0.01	-0.05	0.03	5.58E-01	0.56	-904
Degree of unsaturation[9]	0	-0.05	0.04	8.51E-01	0.46	-693	0	-0.04	0.03	8.12E-01	0.56	-901	-0.01	-0.04	0.03	7.81E-01	0.56	-901	0.00	-0.04	0.04	8.46E-01	0.56	-904
Creatinine[10]	**0.16**	**0.11**	**0.2**	**5.94E-12**	**0.49**	**-740**	**0.07**	**0.03**	**0.12**	**9.42E-04**	**0.56**	**-912**	0.07	0.03	0.11	2.10E-03	0.56	-911	0.06	0.01	0.10	1.37E-02	0.56	-910

Albumin[10]	**0.07**	**0.03**	**0.12**	**1.08E-03**	**0.47**	**-703**	0.04	0	0.08	5.38E-02	0.56	-904	0.04	0.00	0.08	6.70E-02	0.56	-905	0.04	0.00	0.08	6.85E-02	0.56	-907
Alanine[11]	-0.02	-0.06	0.03	4.80E-01	0.46	-693	-0.01	-0.05	0.03	6.29E-01	0.56	-901	-0.01	-0.05	0.03	6.43E-01	0.56	-901	-0.01	-0.05	0.03	6.07E-01	0.56	-904
Glutamine[11]	-0.06	-0.1	-0.01	1.48E-02	0.47	-699	-0.03	-0.08	0.01	9.30E-02	0.56	-903	-0.04	-0.08	0.00	7.46E-02	0.56	-904	-0.04	-0.08	0.00	5.62E-02	0.56	-907
Isoleucine[11]	-0.01	-0.06	0.03	6.33E-01	0.46	-693	-0.02	-0.06	0.02	4.39E-01	0.56	-901	-0.02	-0.06	0.02	4.15E-01	0.56	-902	-0.02	-0.06	0.02	3.97E-01	0.56	-904
Leucine[11]	0.01	-0.04	0.06	7.13E-01	0.46	-693	0	-0.05	0.04	8.55E-01	0.56	-901	-0.01	-0.05	0.04	7.27E-01	0.56	-901	-0.01	-0.06	0.03	5.24E-01	0.56	-904
Valine[11]	0	-0.05	0.04	9.26E-01	0.46	-693	0	-0.04	0.04	9.55E-01	0.56	-901	0.00	-0.04	0.04	9.08E-01	0.56	-901	0.00	-0.04	0.04	9.32E-01	0.56	-904
Phenylalanine[11]	0.04	0	0.09	5.72E-02	0.47	-696	0	-0.04	0.04	9.13E-01	0.56	-901	0.00	-0.04	0.04	9.88E-01	0.56	-901	0.00	-0.04	0.04	8.86E-01	0.56	-904
Tyrosine[11]	-0.06	-0.1	-0.01	1.20E-02	0.47	-699	-0.02	-0.06	0.02	3.78E-01	0.56	-901	-0.02	-0.06	0.02	3.43E-01	0.56	-902	-0.02	-0.06	0.02	3.52E-01	0.56	-904
Histidine[11]	-0.03	-0.07	0.01	1.59E-01	0.47	-695	-0.03	-0.07	0.01	1.26E-01	0.56	-903	-0.03	-0.07	0.01	9.43E-02	0.56	-904	-0.03	-0.07	0.01	9.08E-02	0.56	-906
Glucose[12]	**-0.09**	**-0.13**	**-0.05**	**7.87E-05**	**0.47**	**-708**	-0.04	-0.08	0	4.08E-02	0.56	-905	-0.04	-0.08	0.00	3.91E-02	0.56	-905	-0.04	-0.08	0.00	6.18E-02	0.56	-907
Lactate[12]	-0.02	-0.06	0.02	3.76E-01	0.46	-694	-0.02	-0.06	0.02	3.00E-01	0.56	-902	-0.02	-0.06	0.02	3.26E-01	0.56	-902	-0.02	-0.06	0.02	3.95E-01	0.56	-904
Pyruvate[12]	0	-0.05	0.04	8.81E-01	0.46	-693	-0.01	-0.05	0.03	6.39E-01	0.56	-901	-0.01	-0.05	0.03	7.66E-01	0.56	-901	0.00	-0.04	0.04	9.73E-01	0.56	-904
Citrate[12]	**-0.21**	**-0.25**	**-0.17**	**1.30E-21**	**0.51**	**-784**	**-0.13**	**-0.18**	**-0.09**	**1.91E-10**	**0.57**	**-941**	**-0.14**	**-0.18**	**-0.09**	**1.42E-10**	**0.57**	**-943**	**-0.14**	**-0.18**	**-0.09**	**2.42E-10**	**0.57**	**-944**
Diacylglycerol[12]	-0.03	-0.07	0.01	1.98E-01	0.47	-694	-0.01	-0.05	0.03	5.38E-01	0.56	-901	-0.01	-0.05	0.03	6.01E-01	0.56	-901	-0.01	-0.05	0.03	6.41E-01	0.56	-904
Acetate[13]	-0.01	-0.05	0.03	6.85E-01	0.46	-693	0	-0.04	0.04	8.86E-01	0.56	-901	0.00	-0.04	0.04	8.38E-01	0.56	-901	0.00	-0.04	0.04	8.45E-01	0.56	-904
Acetoacetate[13]	0.03	-0.01	0.07	1.95E-01	0.47	-694	0.01	-0.03	0.05	5.48E-01	0.56	-901	0.01	-0.03	0.05	5.14E-01	0.56	-902	0.01	-0.02	0.05	4.75E-01	0.56	-904
Beta-hydroxybutyrate[13]	-0.01	-0.05	0.03	6.61E-01	0.46	-693	0	-0.04	0.04	8.84E-01	0.56	-901	0.00	-0.04	0.04	9.97E-01	0.56	-901	0.00	-0.04	0.04	9.04E-01	0.56	-904

Table shows regression analyses of 73 serum metabolites versus pQCT derived tibial cortical bone mineral density (BMDC) measured in 1121 participants aged 15.4 (634 females, 487 males). Model 1 = adjustment for age and sex. Model 2 = model 1 in addition to Tanner stage. Model 3 = model 2 in addition to height. Model 4 = model 3 in addition to lean mass and fat mass. β* = SD change in outcome per SD increase in exposure; SE = standard error of β; CI_{95L} = lower 95% confidence estimate of β; CI_{95U} = upper 95% confidence estimate of β; P = strength of evidence against the null hypothesis of no association between the BMDC and serum metabolite; R^2 = variance in outcome explained by the model, AIC = Akaike information criterion. Note: A Bonferroni multiple testing threshold of $P <$ 0.002 (0.05 / 22 principal components) was used to identify metabolites that were robustly associated with BMDC. Observations with sufficient strength of evidence to reject the null hypothesis of no association between the metabolite and BMDC are bolded. Metabolites are classified into the 13 categories, namely: Inflammation[1], Lipoprotein concentration[2], Particle size[3], Apolipoproteins[4], Triglycerides[5], Phospholipids[6], Cholesterol[7], Fatty acids[8],

Fatty acid ratios[9], Fluid balance[10], Amino acids[11], Glycolosis related[12] and Ketone bodies[13]. VLDL = very low density lipoprotein, LDL = low density lipoprotein, IDL = intermediate density lipoprotein, HDL = high density lipoprotein, TG = triglycerides, C = cholesterol and FA = Fatty acids.