Long-term experiment to study the development, interaction and influencing factors of DEXA-parameters

Helmut Fuchs[1], Christine Gau[1], Wolfgang Hans[1], Valerie Gailus-Durner[1] and Martin Hrabe de Angelis[1,2]

[1] Helmholtz Zentrum München, German Mouse Clinic, Institut of Experimental Genetics, German Research Center for Environmental Health (GmbH), Ingolstädter-Landstr. 1, 85764 Neuherberg, Germany.

[2] Technische Universität München, Center of Life and Food Sciences Weihenstephan, Chair of Experimental Genetics, Alte Akademie 8, 85354 Freising, Germany

Running head: DEXA long-term experiment

Corresponding Author:

Martin Hrabe de Angelis

Helmholtz Zentrum München, Institut of Experimental Genetics, German Research Center for Environmental Health (GmbH), Ingolstädter-Landstr. 1, 85764 Neuherberg, Germany. hrabe@helmholtz-muenchen.de, Phone 49-89-3187-3302, Fax 49-89-3187-3500

Abstract

DEXA (dual energy X-ray absorption) is commonly used to measure bone mineral density (BMD), bone mineral content (BMC) and body composition data (fat mass and lean mass) for phenotype assessment in mice. We were interested in the long-term development of bone mineral density, bone mineral content, lean mass and fat mass of mice, also taking into account sex and genetic background. The dataset was used to analyze correlations among the different parameters. We analyzed males and females from two inbred strains C3HeB/FeJ and C57BL/6J starting from 42 until 528 days. To evaluate the effect of husbandry systems, we repeated a part of the study in a second facility with a different caging system. We also assessed different DEXA settings and repeatability of the scans. The results of this study were used to draw conclusions for the use of DEXA analysis in mouse phenotyping approaches.

Introduction

DEXA (dual energy X-ray absorption) analysis is commonly used to assess bone density in human diagnostics for the detection of osteoporosis and bone mineralization defects (Adams 2013, Lorente-Ramos et al. 2012, Blake and Fogelman 2010 and 2009, Crabtree and Ward 2009, Kanis 2002). The same technique is also used in the phenotypic analysis of mutant mice. In both cases, the object is exposed to two distinct X-ray energy levels, whereby the regions of interest are then systematically scanned. DEXA takes advantage of the different X-ray absorption of tissues in order to discriminate between bone, lean mass and fat mass (Sorenson et al. 1989, Srivastava et al. 2003, Nagy and Clair 2000, Lochmüller et al. 2001, Akhter et al. 2004, Andreoli et al. 2009).

DEXA is one standard technology for diagnosis of osteoporosis. DEXA scans are quick and easy to perform and have additional advantages such as the low X-ray dose needed for the analysis and its suitability for whole body analysis in mouse models. Its disadvantages include its area-based output (BMD is measured as mass per area [g/cm²] instead of mass per volume, as in computed tomography) and its inability to discriminate between cortical and trabecular bone fractions (Fuchs et al. 2006).

DEXA technology is useful for the assessment of bone mineral density and body composition in many large-scale mouse phenotyping approaches (e.g. the International Mouse Phenotyping Consortium, IMPC, Brown and Moore 2012, www.mousephenotype.org, or the German Mouse Clinic, www.mouseclinic.de, Gailus-Durner et al. 2005, Gailus-Durner et al. 2009, Fuchs et al. 2011, Fuchs et al. 2012). Despite its limitations (new DEXA devices are no longer commercially available, unless sourced as refurbished units), there are no alternatives for mouse clinic approaches and the use in high throughput screens at the moment, as other technologies, such as peripheral quantitative computed tomography (pQCT) or micro-computed-tomography (micro-CT), are more time consuming and are less automated.

DEXA apparatuses are either based on the scan of the transmitted X-ray energy through the radiated tissue, whereby an X-ray sensitive scanner scans line per line the defined area beneath the target tissue (used for example by the pDEXA Sabre system supplied by Stratec Medizintechnik). Another type of DEXA technology applies a cone beam in combination with an X-ray sensitive camera (used for example in the Lunar PIXImus system supplied by GE Medical Systems). The advantage of this technique, which analyses only the mouse body, is the quick mode of analysis. A special feature of the first mentioned line based pDEXA Sabre system is that the analysis of the scanned data can be performed in either an automated way or by entering a histogram average width (HAW) value, which means a threshold for the discrimination of tissues.

A significant amount of literature is currently available on the reliability of DEXA analysis in mice (Nagy and Clair 2000) as well the influence of sex and genetic background on murine DEXA parameters (Beamer et al. 2002; Akhter et al. 2004; Orwoll et al. 2001). However, no study has evaluated the influences and correlations of the factors age, weight, sex, genetic background and animal husbandry system on DEXA parameters over the entire life span of the mouse. We aimed to monitor the development of weight and the data derived from DEXA analysis, bone mineral density (BMD), bone mineral content (BMC), fat mass and lean mass in mice over a 1.5 years period taking into consideration the influence of sex and genetic background, the latter represented by two inbred strains, C3HeB/FeJ and C57BL/6J. The two strains were selected as they represent two inbred mouse strains and that are known for differences in bone mineral density and bone mineral content (Beamer et al. 1996, Beamer et al. 2002). They are genetically distant from each other and represent a major fraction of the genetic variability within mouse strains. Both strains are quite frequently used for different

studies (C57BL/6 substrains are frequently used for knock-out studies, C3H substrains are for example used for ENU mutagenesis , e.g. Hrabe de Angelis et al. 2000, Nolan et al. 2000). In addition, we were interested in the influences of husbandry effects as well as the investigation of the correlations between the different factors. Furthermore, repeatability and two optional analysis settings of the used DEXA machine should be investigated.

Material and Methods

Study design

In order to analyse the development of bone mineral density (BMD), bone mineral content (BMC), lean mass and fat mass in a long-term experiment under consideration of the influences age, weight, sex, genetic background, and husbandry conditions, male and female mice from two inbred mouse strains C3HeB/FeJ and C57BL/6J were aged in two different mouse facilities (facility G and D) of the Helmholtz Zentrum München, and were analysed at several times by DEXA scans. Each group consisted of 10 animals. The animals in facility D were measured 4 times in the interval between 64 and 264 days and animals from facility G were measured 8 times in the interval between 42 and 528 days (see Table 1). The time of analysis differed according to availability of machine and measurement capacities. Some animals could not be analysed throughout the complete experimental period. The housing conditions in facility D were conventional type II cages (267x208x149mm, UNO, Netherlands) with filter tops. Mice in facility G were housed in type II individually ventilated cages (IVC, BioZoneGlobal, Ramsgate, Kent, UK). The mice in both facilities had access to food (Altromin 1314, Lage, Germany, dry matter, 89%, crude protein 22.5%, crude fat 5.0%, crude fibre 4.5%, crude ash 6.5%, NfE 50.5%, metaboliazable energy 12.5 MJ/kg) and water ad libitum. A detailed comparison of the housing conditions in both facilities is shown in Table 2. Due to quarantine reasons separate DEXA machines had to be used for each facility. By exchanging the calibration phantoms the comparability of the two systems was confirmed (for BMD and BMC measurements the results did not differ more than 3%).

To analyse measurement repeatability, mice in facility D were scanned twice: They were placed on the testing surface, scanned, then removed from the DEXA scanner and

subsequently placed again in the machine and scanned a second time. As for the used DEXA system there is the possibility to do either an automated analysis of the data by the software system or to run the analysis by setting manually a HAW (Histogram Averaging Width) value, the data from the two scans from facility D has been analysed under both settings. Data from facility G was only analysed under a constant manually set HAW value of 0.020.

DEXA analysis

Bone mineral density (BMD), bone mineral content (BMC), fat mass and lean mass were measured in anesthetized mice with the pDEXA Sabre X-ray Bone Densitometer (Norland Medical Systems Inc., Basingstoke, Hampshire, UK; distributed by Stratec Medizintechnik GmbH, Pforzheim, Germany). The Histogram Averaging Width (HAW) was set to either to automated analysis or 0.020 (according to the recommendations in the user's manual). Scan speed was 20 mm/s, and resolution was 0.5 mm x 1.0 mm. The X-ray dose that a mouse was exposed to was 300 µSv per scan (according to the manufacturer's information). The system was calibrated on a daily basis according to the manufacturer's recommendations using the QC and QA phantoms. Measure procedure: After anaesthesia was administered (0,1 g ketamine and 0,01 g xylazine per kg body weight), the weight of each mouse was recorded, and the mouse was then placed in the DEXA scanner. After a scout scan, the area of interest was optimized and the measure scan was started. For data analysis, a region comprising the entire body of the mouse was defined.

Mice

Wild type mice of inbred strains C3HeB/FeJ and C57BL/6J were used for this study. Founder stocks of C3HeB/FeJ and C57BL/6J mice were directly imported from the Jackson Laboratory, and bred and grown in the animal facilities of the Helmholtz Zentrum München. The mice used for this study were between 5 to 10 generations apart from the original Jackson substrain. All animal experiments were done according to the German laws for animal protection and by permission of the Regierung von Oberbayern.

Data analysis

Scatterplots were used to visualize the data, where a data point for each mouse at every time point and a cubic smoothing spline for each subgroup (as given in Chambers and Hastie 1992, using a smoothing parameter of 0.9 and 2 degrees of freedom) is produced to give an impression of the general differences between the subgroups.

For the comparison of facilities G and D a Linear Mixed-Effects Model was used (fit by maximizing the restricted log-likelihood, see Laird and Ware 1982 for details). The used dataset included all measurements of both facilities until the age of 300 days.

All statistical analysis were conducted with R (R Development Core Team 2009).

Results

Eight groups of mice (consisting of either 10 male or 10 female mice from mouse strains C3HeB/FeJ or C57BL/6J) were housed in two different facilities. Each mouse was regularly tested for body weight, BMD and BMC as well as body composition parameters fat mass and lean mass (Table 1). The study covered a period of approximately 1.5 years.

Development of body weight, bone mineral density, bone mineral content, fat mass and lean mass

We were interested in the development of bone and body composition parameters of male and female mice of different genetic background over the complete experimental period. For the awareness of influences of different housing conditions, the experiment was run separately in two independent facilities with different husbandry systems. In Fig. 1 the development of body weight over time of male and female C3HeB/FeJ (Fig. 1a) and C57BL/6J (Fig. 1b) mice from facility D and G is shown. In facility G, for both strains males are heavier than females in the first two hundred days of life. In C3HeB/FeJ mice, in the following period the female animals gained more weight, and were finally on the same level as males. In C57BL/6J mice, the weight difference between males and females persisted also in later phases of the study. The number of C57BL/6J females that were available for the final measurements was decreased. So the mean weight gain of the cohort has to be considered with caution, as the remaining animals were the ones that had previously higher body weights (Fig. 1b, shown as dotted line). In facility D, the findings were consistent for the covered period of investigation, but all groups were much lighter than the corresponding ones from facility G (the same applies in general for the data from the other parameters where we focus mostly on the presentation of the data from facility G).

The initial measurement of fat mass in many of the animals was below the detection limit of the DEXA system. Fat mass increased in all groups over the first 200-300 days, and then plateaued. The data for female C57BL/6J mice deviated from this finding, possibly due to the small number of animals available for the final measurement. In C3HeB/FeJ mice older than 100 days, females had higher fat mass compared to their male counterparts. In C57BL/6J

mice, males had higher fat mass than females until approximately 300 days. Thereafter, the same trend as noted previously for the C3HeB/FeJ strain was found, i.e., that older females had higher fat mass than males of the same age. For the remaining females (N = 4) at 528 days, the highest fat values were found. The variation in the fat mass increased significantly in all mouse groups with increasing age (Fig. 2a). In contrast to the finding of a considerable increase in fat mass over time, no common trend was found for lean mass among the groups analyzed. In addition, the variation in the lean mass data appeared to increase with age (Fig. 2b).

BMC as well as BMD increased remarkably within the first 170 days of life in animals of both sexes and strains. After this period a moderate but steady increase in BMC and BMD was observed in all C3HeB/FeJ mice regardless of sex (Fig. 2c and d). While in C3HeB/FeJ curves for males and females are nearly identical over the whole experimental period, in C57BL/6J the males showed higher values until day 220, and then there was a stagnation for both, BMC and BMD.

Correlations among different parameters

The collected data is perfectly suited to gain information about correlations among the different parameters. In a first step, we related all measured parameters to body weight. In a next step, correlations among various parameters were identified by the creation of a scatterplot matrix for BMD, BMC, lean mass, fat mass, soft tissue and body weight data.

Fat mass, lean mass, BMC and BMD related to body weight

Most parameters derived from DEXA analysis are confounded by body weight. In Fig. 3, fat mass, lean mass, BMC and BMD are plotted against body weight. The higher the body weight, the higher the fat mass, BMC and BMD (for C57BL/6J strain, peak bone density occurred at approximately 40 g). No obvious correlation was found between lean mass and body weight.

With increasing body weight, the variation in DEXA parameters increased. It was not clear whether this observation reflected a biological variation, or whether this finding was, in part,

influenced by a decrease in the accuracy of the DEXA measurement in mice with higher body weights.

Further relations between DEXA parameters

The data set that we collected within this study contains the potential to obtain information about the relations between the parameters that have been analyzed. To analyze the relationships among the DEXA parameters, we created a scatterplot matrix (Fig. 4) where BMD, BMC, lean mass, fat mass, soft tissue (soft tissue = fat mass + lean mass) and body weight were plotted against each other. Plots of body weight, soft tissue, and fat mass vs. BMC were highly linear. The strongest relation was noted for fat mass vs. BMC. A clear linear relation between soft tissue and body weight was also noted. Lean mass showed no relation with any of the other parameters.

Influence of facility and housing conditions

In order to obtain first information about the influence of housing conditions, the first part of the experiment was conducted in two different facilities with different husbandry systems. The influence of the facility was analyzed by application of a linear mixed-effects model fit by REML (Table 3) where we considered only the parameter body weight. As the age range used for data collection was greater in facility G compared to facility D, weight data that corresponded to an age over 300 days was excluded from the analysis. A significant influence of the housing conditions was demonstrated ($p < 0.001$). All estimated values of the linear mixed-effects model were interpreted using the reference categories (facility G, sex f, strain C3HeB/FeJ). The intercept showed the estimated value for weight at an age of zero of a female C3HeB/FeJ animal housed in facility G. The average animal gained every day 0.085 g of weight per day. The weight of animals housed in facility D was 5.342 less than in facility G (if all other covariates are equal). A male mouse was in average 6.179 g heavier than a female mouse. A C57BL/6J-mouse is in average 7.549 g lighter than a C3HeB/FeJ mouse of the same sex, housed in the same facility and of the same age.

Repeatability of DEXA scans and comparison of analysis settings

In order to obtain information about the repeatability of DEXA measurements, we performed each scan from the mice from facility D at each time point with one repetition. The mice were anaesthetized, put on the measurement platform, scanned for the first scan, removed from the platform, and placed again for the second scan. For visualization of the resulting data, values from the first scan are plotted versus the second scan (Fig. 5 a and b).

The pDEXA Sabre system offers different options for the analysis of the scanned data set. A HAW (Histogram Averaging Width) value for the differentiation of the different tissues can be either entered manually, or the system calculates automatically the best value for each single scan. For the data set obtained from facility D, we analysed each of the two scans by two different settings (automated selection and a fixed value of 0.020 for HAW) and compared the obtained results. As a measure for the goodness of the analysis, we calculated the correlation coefficient for the first scan versus the second scan under both settings. The correlation coefficients for the alternative settings are summarized in Table 4. For the bone parameters the manual setting revealed higher values than the automated analysis (0.932 versus 0.842 for BMC and 0.816 versus 0.700 for BMD). For lean and fat mass, it was just the other way round (0.922 versus 0.903 for fat mass and 0.872 versus 0.840 for lean mass), but the difference between the alternative settings was not as pronounced.

Discussion

We studied the development of, and relationships between DEXA derived parameters fat mass, lean mass, bone mineral content, bone mineral density and body weight to each other in the framework of a long-term experiment. If data is correlated to each other, and only a limited life period of a mouse life is considered for the analysis, the data will be considered as more or less linearly related. The data that we collected within this study covers a long period within the life of mice, and delivers more detailed information about the relation of parameters (e.g. which phases represent linear relations between parameters and which do not).

As shown in Fig. 2, a considerable increase in fat mass, BMC, and BMD occurred within the first 100 days of life. These findings suggest that age has a strong impact on these DEXA-derived parameters when the measurements are taken at a young age. This fact may have consequences for the use of DEXA in phenotyping activities where only a single analysis is planned or possible (for example, in international large scale phenotyping projects like EUMODIC, www.eumodic.org; Morgan et al. 2010; Ayadi et al. 2012; or the IMPC, Brown and Moore 2012, www.mousephenotype.org). In addition, greater variation in the data was found as the mice aged. According to our data, the increase in variation with the age of the animals was only partly due to technical limitations of the DEXA technology, and seemed to primarily reflect the biological situation. This finding is important for the design of aging studies. A balance between the effect of aging and the increased variation among the data is required. Based on our results, the optimal timing for such measurements appears to occur at approximately 15 to 20 weeks of age.

For female C57BL/6J mice we observed a strong reduction of lean mass with time (Figures 2b and 3b). We do not expect that there is a decrease in lean mass, at least not as drastic as observed in the measured data. There were too few mice in this group to be able to draw specific conclusions. We only speculated that a fraction of the lean mass might have been interpreted as fat mass by the system.

Peak bone mass

The two mouse strains selected for this study were chosen because they represent the extremes in murine bone density values. The C3HeB/FeJ strain is known to have a higher bone density than the C57BL/6J strain which is known to have a low bone density (Beamer et al. 1996 and 2002, Richman et al. 2001). Regarding BMC and BMD we were interested in the peak bone mass and peak bone density, which could not be identified within our data for C3HeB/FeJ mice. For C57BL/6J males there is a maximum for BMD at 40 g or 200 days, respectively. In C57BL/6J females, BMC increased continuously according to body weight in a manner similar to that seen in C3HeB/FeJ mice. BMD plateaued in the C57BL/6J females at about the same age as the C57BL/6J males achieved their peak bone mass (Fig. 3). Data for C57BL/6J females in the final phase of the experiment was based on only four animals, and, therefore, the data may be biased. For both strains, females show higher BMC and BMD values in relation to body weight compared to the males. Beamer et al. (1996) performed pQCT analysis on different inbred mouse strains and the greatest difference in femur density was found between C57BL/6J and C3H/HeJ females, with the C3H/HeJ femur density nearly 50% greater. They analysed also total femur density by age and found that there are strain differences as early as 2 months of age and the bone density levels acquired in adulthood were consistently maintained through 12 months.

In Fig. 2, fat mass, lean mass, BMC, and BMD are shown vs. age of the mice. For the C57BL/6J mice, the graphs of older animals showed some unexpected variations, especially with regards to BMC and BMD. If the data, however, was plotted vs. weight instead of age, steady increases in fat mass, BMC, and BMD were noted. We concluded that body weight had a stronger confounding influence than the age on DEXA parameters of the mice. The older the mice are, the better this gets visible, and the influences of age and weight diverge (reflected by different graphs of these parameters when plotted against age and weight, respectively, in Fig. 2 and Fig. 3).

We plotted all parameters against each other (Fig. 4) to test for relations among the variables. Many of the variables (e.g., body weight vs. soft tissue mass, body weight vs. BMC, soft tissue mass vs. BMC, and fat mass vs. BMC) showed a linear relation over the entire time period, whereas other combinations demonstrated a nonlinear relation (e.g., body weight vs. BMD). Lean mass was the only parameter that showed no relation with any of the other

parameters. We speculate that the reason for this observation might be the high degree of variation within this parameter or possibly technical problems of the DEXA system encountered in correctly differentiating lean mass from fat mass.

With Fig. 4 we intended to give an overview about the different relations of the parameters to each other. The strength of our dataset is that it covers a long period in the life of mice. In studies with shorter intervals most parameters might show more or less linear relations. In the case of our dataset there is the possibility to differentiate between linear and non-linear relations.

Influence of facility and housing conditions

As there is a lot of discussion within the scientific community about the comparability of data between different institutions (e.g. for the use in international large scale phenotyping projects like EUMODIC, www.eumodic.org, Morgan et al. 2010, Ayadi et al. 2012 or IMPC, Brown and Moore 2012, www.mousephenotype.org) we were interested in the development of DEXA parameters within the same institution but in two different facilities with different husbandry systems. The first part of the experiment (up to day 264) was performed independently in two different facilities using different cage systems and climate controlling technology. Mice in facility D were kept in conventional type II cages with filter tops. In facility G the mice were housed in type II individually ventilated cages (IVC, BioZoneGlobal). Using the body weight data we applied a linear mixed-effects model fit by REML and found that the type of facility had a significant influence on DEXA parameters. From Fig. 1a and Fig. 1b, higher values in body weight for all groups housed in facility G were noted when compared with facility D. DEXA scans in facility D were carried out twice for each animal at each time point for assessment of the repeatability of the scans. Thus, there was a difference in the experimental setup between the mice from the two facilities. It might be argued that this step could have caused additional stress on the mice in facility D resulting in reduced weight gain, although this is unlikely based on our observations of the animals' conditions. We speculate that the differences in body weight might be due to a different micro-climate inside the cages. Animal caretakers reported higher temperatures and humidity in the IVC cages compared to conventional cages with filter tops. Quantification of these observations unfortunately failed. Given a mutation rate of around 100 SNPs per generation

(Lynch 2010) further differences between the data obtained in the two different facilities might be due to a slightly different genetic history of the strains that were analyzed in the two facilities: The mice were imported directly from the Jackson Laboratory to a central core breeding unit, but the breeding for the cohort preparation for the study was done independently for at least two generation in the two separate facilities. Another source for the differences of the data obtained from the two facilities might come from a changed microbiome in the mice of the different facilities (Maynard et al. 2012, Turnbaugh et al. 2006). The analysis of this additional factor was out of the scope of our study, but from the literature there is for example information that gut flora influences the fat content in mice (Turnbaugh et al. 2006), or the behavior (Neufeld et al. 2011) which then might have secondary effects on the analyzed parameters in our study.

Repeatability and HAW setting

We analyzed the data set from facility D with two different settings in the analysis software that is implemented in the scanner system. The two scans were taken just one after the other. After the scan was taken, and the data was stored in the machine, each data-set was analyzed once using the automated algorithm and once using the fixed setting. According to the user's manual a Histogram Averaging Width (HAW) can be selected. There is the option to either use an analysis that is performed in an automated way by the software system from the DEXA device. In this case, the data is analyzed for the best fit of threshold values within the scan data for each single scan. The other option is the use of fixed thresholds for the analysis. We used a HAW (Histogram Averaging Width) value of 0.020 that was recommended by the manufacturer for bone analysis. The advantage of the automated analysis option is that the data of each scan is analyzed using analysis parameters that fit best for the acquired data. However, for studies where many datasets from different individuals have to be analyzed and compared, this means that every scan is treated differently, and the direct comparison of the results has to be considered with caution.

We calculated correlation coefficients for scan 1 vs. scan 2 for each of the settings and compared the obtained values as a measure for the best way of analysis. For the bone parameters BMD and BMC, the fixed setting of HAW to 0.020 revealed better correlations between the two scans. For lean and fat mass the correlation was higher using the automated

setting. As the difference in lean and fat mass was not as pronounced as the difference of BMC and BMD between the two optional settings, we decided to use the HAW setting of 0.020 for all further analysis. This reflects also our aim of a standardized analysis where all mice within the study, and all data that we collected from them, are analyzed under the same conditions. This decision would allow a better cross comparison between the data of single mice.

The high accuracy of the DEXA technology is reflected in the plot of soft tissue vs. body weight (Fig. 4). Data points in the plot are linear and almost totally independent from sex, strain, and age. This suggests that the system is able to calculate the body weight of a mouse in an exact manner (body weight = lean mass + fat mass + constant). However, the discrimination of soft tissue into lean mass and fat mass might be difficult. For example, for the female C57BL/6J mice we measured a decrease in lean mass over the total experiment. As the mice were still growing, the system must have falsely interpreted the body composition data. Brommage (2003) employed carcass analysis to determine the accuracy of the PIXImus2 DEXA system in measuring body fat in mice by using acetone for fat extraction. The PIXImus2 overestimated mouse body fat by ~3.3 g which was similar to findings by Nagy and Clair (2000), in which diethyl ether was employed for fat extraction. Also Lochmüller et al. (2001) demonstrated a good precision of bone and moderate precision of body composition measurements in small animals, using a high-resolution DEXA system. Comparison of lean mass and fat mass information derived from other methods such as nuclear magnet resonance (NMR) also resulted in numbers that were different for lean mass and fat mass than those obtained using DEXA (e.g. Abe et al. 2011). However, the high degree of linearity in the plot of soft tissue versus body weight reflects that soft tissue was evaluated with a very high accuracy. Halldorsdottir et al. 2009 compared DEXA (PIXImus) and time domain nuclear magnetic resonance (Bruker Optics) for the measurement of body composition. They found that DEXA consistently overestimated lean mass and fat mass by ~8% and ~46%, respectively, while NMR only slightly underestimated lean mass by ~0.2% and overestimated fat mass by ~15%.

Conclusions

DEXA technology is a useful tool for the assessment of body composition and bone density in mice. It is quick and reliable, but has certain limitations. Measurements of fat mass were in very young animals below the detection limits of the system. As a conclusion we implemented a minimum body weight of 18g for all DEXA measurements performed per our protocol. The DEXA scanner has high accuracy, but discrimination between fat and lean mass can fail under certain circumstances. Special care should be taken regarding the settings of the scanner: if the focus of the measurement is on fat and lean mass, we recommend using the automated analysis modus, for bone analysis a fixed HAW setting of 0.020 was suitable. If only one measurement is planned to characterize a mutant mouse line with DEXA, it should be carried out in the age range between 15 and 20 weeks. Younger mice are still growing, and there is a strong influence of the age and developmental status of single animals on the measurements. In older animals the variation in measurements is higher. According to our data we observed in the age between 15 to 20 weeks the best combination of only a low to moderate influence of the age of the mice on the expected DEXA parameters, and the variation of each measured parameter due to biological and/or technical variation. We could not clearly assign a peak bone mass. In the high bone density strain C3HeB/FeJ, bone density increased over the complete experimental period. There are many confounding factors on DEXA parameters that have to be taken into consideration when analyzing the results. Environmental conditions reflected by animal housing conditions have an impact on the measured values. There might be influences on the data resulting from the type of DEXA device that is used. The data of this work is only based on the use of one system. In summary, DEXA is a robust technology for mouse phenotyping as long as all influencing factors are taken into consideration.

Acknowledgements

We would like to thank Reinhard Seeliger, Susanne Wittich, and Gregor Pahnke, for their technical assistance. In addition, we wish to express our thanks to Monika Denk and Horst Wenig, as well as the entire GMC animal caretaker team, for their exceptional animal care. This work was supported by Infrafrontier BMBF (grant number 01KX1012).

References

Abe K, Fuchs H, Boersma A, Hans W, Yu P, Kalaydjiev S, Klaften M, Adler T, Calzada-Wack J, Mossbrugger I, Rathkolb B, Rozman J, Prehn C, Maraslioglu M, Kametani Y, Shimada S, Adamski J, Busch DH, Esposito I, Klingenspor M, Wolf E, Wurst W, Gailus-Durner V, Katan M, Marschall S, Soewarto D, Wagner S, Hrabe de Angelis M (2011) A novel N-ethyl-N-nitrosourea-induced mutation in phospholipase Cγ2 causes inflammatory arthritis, metabolic defects, and male infertility in vitro in a murine model. Arthritis Rheum. 63(5):1301-1311

Adams JE (2013) Advances in bone imaging for osteoporosis. Nat Rev Endocrinol 9(1):28-42

Akhter MP, Otero JK, Iwaniec UT, Cullen DM, Haynatzki GR, Recker RR (2004) Differences in vertebral structure and strength of inbred female mouse strains. J Musculoskelet Neuronal Interact 4(1):33-40

Andreoli A, Scalzo G, Masala S, Tarantino U, Guglielmi G (2009) Body composition assessment by dual-energy X-ray absorptiometry (DXA). Radiol Med. 2009 Mar;114(2):286-300.

Ayadi A, Birling MC, Bottomley J, Bussell J, Fuchs H, Fray M, Gailus-Durner V, Greenaway S, Houghton R, Karp N, Leblanc S, Lengger C, Maier H, Mallon AM, Marschall S, Melvin D, Morgan H, Pavlovic G, Ryder E, Skarnes WC, Selloum M, Ramirez-Solis R, Sorg T, Teboul L, Vasseur L, Walling A, Weaver T, Wells S, White JK, Bradley A, Adams DJ, Steel KP, Hrabě de Angelis M, Brown SD, Herault Y (2012) Mouse large-scale phenotyping initiatives: overview of the European Mouse Disease Clinic (EUMODIC) and of the Wellcome Trust Sanger Institute Mouse Genetics Project. Mamm Genome 23(9-10):600-610

Beamer WG, Donahue LR, Rosen CJ, Baylink DJ (1996) Genetic variability in adult bone density among inbred strains of mice. Bone 18(5):397-403

Beamer WG, Donahue LR, Rosen CJ (2002) Genetics and bone using the mouse to understand man. J Musculoskelet Neuronal Interact. 2(3):225-231

Becker RA, Chambers JM, Wilks AR (1988) The New S Language. Wadsworth & Brooks/Cole

Blake GM, Fogelman I (2009) The clinical role of dual energy X-ray absorptiometry. Eur J Radiol 71(3):406-414

Blake GM, Fogelman (2010) An update on dual-energy x-ray absorptiometry. Semin Nucl Med 40(1):62-73

Brommage R. (2003) Validation and calibration of DEXA body composition in mice. Am J Physiol Endocrinol Metab.285(3):E454-9

Brown SD, Moore MW (2012) The International Mouse Phenotyping Consortium: past and future perspectives on mouse phenotyping. Mamm Genome 23(9-10):632-640

Chambers JM and Hastie TJ (1992) Statistical Models in S. Wadsworth & Brooks/Cole

Crabtree N, Ward K (2009) Bone densitometry: current status and future perspectives. Endocr Dev 16:58-72

Fuchs H, Lisse T, Abe K and Hrabe de Angelis M (2006) Screening for bone and cartilage phenotypes in mice. In: Hrabe de Angelis M, Chambon P, Browns S (eds) Phenotyping of the Laboratory Mouse Wiley-VCH, Weinheim, pp 35-86

Fuchs H, Gailus-Durner V, Adler T, Aguilar-Pimentel JA, Becker L, Calzada-Wack J, Da Silva-Buttkus P, Neff F, Götz A, Hans W, Hölter SM, Horsch M, Kastenmüller G, Kemter E, Lengger C, Maier H, Matloka M, Möller G, Naton B, Prehn C, Puk O, Rácz I, Rathkolb B, Römisch-Margl W, Rozman J, Wang-Sattler R, Schrewe A, Stöger C, Tost M, Adamski J, Aigner B, Beckers J, Behrendt H, Busch DH, Esposito I, Graw J, Illig T, Ivandic B, Klingenspor M, Klopstock T, Kremmer E, Mempel M, Neschen S, Ollert M, Schulz H, Suhre K, Wolf E, Wurst W, Zimmer A, Hrabě de Angelis M (2011) Mouse phenotyping. Methods 53(2):120-135

Fuchs H, Gailus-Durner V, Neschen S, Adler T, Afonso LC, Aguilar-Pimentel JA, Becker L, Bohla A, Calzada-Wack J, Cohrs C, Dewert A, Fridrich B, Garrett L, Glasl L, Götz A, Hans W, Hölter SM, Horsch M, Hurt A, Janas E, Janik D, Kahle M, Kistler M, Klein-Rodewald T, Lengger C, Ludwig T, Maier H, Marschall S, Micklich K, Möller G, Naton B, Prehn C, Puk O, Rácz I, Räss M, Rathkolb B, Rozman J, Scheerer M, Schiller E, Schrewe A, Steinkamp R,

Stöger C, Sun M, Szymczak W, Treise I, Vargas Panesso IL, Vernaleken AM, Willershäuser M, Wolff-Muscate A, Zeh R, Adamski J, Beckers J, Bekeredjian R, Busch DH, Eickelberg O, Favor J, Graw J, Höfler H, Höschen C, Katus H, Klingenspor M, Klopstock T, Neff F, Ollert M, Schulz H, Stöger T, Wolf E, Wurst W, Yildirim AÖ, Zimmer A, Hrabě de Angelis M (2012) Innovations in phenotyping of mouse models in the German Mouse Clinic. Mamm Genome 23(9-10):611-622

Gailus-Durner V, Fuchs H, Becker L, Bolle I, Brielmeier M, Calzada-Wack J, Elvert R, Ehrhardt N, Dalke C, Franz TJ, Grundner-Culemann E, Hammelbacher S, Holter SM, Holzlwimmer G, Horsch M, Javaheri A, Kalaydjiev SV, Klempt M, Kling E, Kunder S, Lengger C, Lisse T, Mijalski T, Naton B, Pedersen V, Prehn C, Przemeck G, Racz I, Reinhard C, Reitmeir P, Schneider I, Schrewe A, Steinkamp R, Zybill C, Adamski J, Beckers J, Behrendt H, Favor J, Graw J, Heldmaier G, Hofler H, Ivandic B, Katus H, Kirchhof P, Klingenspor M, Klopstock T, Lengeling A, Muller W, Ohl F, Ollert M, Quintanilla-Martinez L, Schmidt J, Schulz H, Wolf E, Wurst W, Zimmer A, Busch DH, de Angelis MH (2005) Introducing the German Mouse Clinic: open access platform for standardized phenotyping. Nat Methods 2(6):403-404

Gailus-Durner V, Fuchs H, Adler T, Aguilar Pimentel A, Becker L, Bolle I, Calzada-Wack J, Dalke C, Ehrhardt N, Ferwagner B, Hans W, Hölter SM, Hölzlwimmer G, Horsch M, Javaheri A, Kallnik M, Kling E, Lengger C, Mörth C, Mossbrugger I, Naton B, Prehn C, Puk O, Rathkolb B, Rozman J, Schrewe A, Thiele F, Adamski J, Aigner B, Behrendt H, Busch DH, Favor J, Graw J, Heldmaier G, Ivandic B, Katus H, Klingenspor M, Klopstock T, Kremmer E, Ollert M, Quintanilla-Martinez L, Schulz H, Wolf E, Wurst W, Hrabe de Angelis M (2009) Systemic first-line phenotyping. Methods Mol Biol 530:463-509

Halldorsdottir S, Carmody J, Boozer CN, Leduc CA, Leibel RL (2009) Reproducibility and accuracy of body composition assessments in mice by dual energy x-ray absorptiometry and time domain nuclear magnetic resonance. Int J Body Compos Res. 7(4):147-154

Hrabé de Angelis MH, Flaswinkel H, Fuchs H, Rathkolb B, Soewarto D, Marschall S, Heffner S, Pargent W, Wuensch K, Jung M, Reis A, Richter T, Alessandrini F, Jakob T, Fuchs E, Kolb H, Kremmer E, Schaeble K, Rollinski B, Roscher A, Peters C, Meitinger T, Strom T, Steckler T, Holsboer F, Klopstock T, Gekeler F, Schindewolf C, Jung T, Avraham K, Behrendt H, Ring J, Zimmer A, Schughart K, Pfeffer K, Wolf E, Balling R (2000)

Genome-wide, large-scale production of mutant mice by ENU mutagenesis. Nat Genet. 2000 Aug;25(4):444-7

Kanis JA (2002) Diagnosis of osteoporosis and assessment of fracture risk. Lancet 359(9321):1929-1936

Laird NM and Ware JH (1982) Random-Effects Models for Longitudinal Data. Biometrics 38: 963-974

Lynch (2010) Evolution of the mutation rate. Trends in Genetics 26 (2010) 345–352

Lochmüller EM, Jung V, Weusten A, Wehr U, Wolf E, Eckstein F (2001) Precision of high-resolution dual energy X-ray absorptiometry of bone mineral status and body composition in small animal models. Eur Cell Mater 1:43-51

Lorente Ramos RM, Azpeitia Armán J, Arévalo Galeano N, Muñoz Hernández A, García Gómez JM, Gredilla Molinero J (2012) Dual energy X-ray absorptiometry: fundamentals, methodology, and clinical applications. Radiologia 54(5):410-423

Maynard CL, Elson CO, Hatton RD, Weaver CT. (2012) Reciprocal interactions of the intestinal microbiota and immune system. Nature 489(7415):231-41

Morgan H, Beck T, Blake A, Gates H, Adams N, Debouzy G, Leblanc S, Lengger C, Maier H, Melvin D, Meziane H, Richardson D, Wells S, White J, Wood J; EUMODIC Consortium, de Angelis MH, Brown SD, Hancock JM, Mallon AM (2010) EuroPhenome: a repository for high-throughput mouse phenotyping data. Nucleic Acids Res 38(Database issue):D577-585 doi: 10.1093/nar/gkp1007. Epub 2009 Nov 23.

Nagy TR, Clair AL (2000) Precision and accuracy of dual-energy X-ray absorptiometry for determining in vivo body composition of mice. Obes Res 8(5):392-398

Neufeld KM, Kang N, Bienenstock J, Foster JA (2011) Reduced anxiety-like behavior and central neurochemical change in germ-free mice. Neurogastroenterol Motil.23(3):255-64

Nolan PM, Peters J, Strivens M, Rogers D, Hagan J, Spurr N, Gray IC, Vizor L, Brooker D, Whitehill E, Washbourne R, Hough T, Greenaway S, Hewitt M, Liu X, McCormack S, Pickford K, Selley R, Wells C, Tymowska-Lalanne Z, Roby P, Glenister P, Thornton C, Thaung C, Stevenson JA, Arkell R, Mburu P, Hardisty R, Kiernan A, Erven A, Steel KP,

Voegeling S, Guenet JL, Nickols C, Sadri R, Nasse M, Isaacs A, Davies K, Browne M, Fisher EM, Martin J, Rastan S, Brown SD, Hunter J (2000) A systematic, genome-wide, phenotype-driven mutagenesis programme for gene function studies in the mouse. Nat Genet. 2000 Aug;25(4):440-3

Orwoll ES, Belknap JK, Klein RF (2001) Gender specificity in the genetic determinants of peak bone mass. J Bone Miner Res 16(11):1962-1971

R Development Core Team (2009) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL http://www.R-project.org

Richman C, Kutilek S, Miyakoshi N, Srivastava AK, Beamer WG, Donahue LR, Rosen CJ, Wergedal JE, Baylink DJ, Mohan S (2001) Postnatal and pubertal skeletal changes contribute predominantly to the differences in peak bone density between C3H/HeJ and C57BL/6J mice. J Bone Miner Res 16(2):386-397

Sorenson JA, Duke PR, Smith SW (1989) Simulation studies of dual-energy X-ray absorptiometry. Med Phys 16:75-80

Srivastava AK, Mohan S, Wergedal JE, Baylink DJ (2003) A genomewide screening of N-ethyl-N-nitrosourea-mutagenized mice for musculoskeletal phenotypes. Bone 33(2):179-191

Turnbaugh PJ, Ley RE, Mahowald MA, Magrini V, Mardis ER, Gordon JI. (2006) An obesity-associated gut microbiome with increased capacity for energy harvest. Nature 444(7122):1027-31

Tables

Table 1. Study design

<table>
<tr><td colspan="4">Facility D</td><td colspan="4">Facility G</td></tr>
<tr><td colspan="2">C3HeB/FeJ</td><td colspan="2">C57BL/6J</td><td colspan="2">C3HeB/FeJ</td><td colspan="2">C57BL/6J</td></tr>
<tr><td>Males</td><td>Females</td><td>Males</td><td>Females</td><td>Males</td><td>Females</td><td>Males</td><td>Females</td></tr>
<tr><td>10 mice</td><td>10 mice</td><td>10 mice</td><td>10 mice</td><td>10 mice</td><td>10 mice</td><td>10 mice</td><td>10 mice</td></tr>
<tr><td colspan="4">Analysis by DEXA 4 times in the interval between 64 and 264 days</td><td colspan="4">Analysis by DEXA 8 times in the interval between 42 and 528 days</td></tr>
<tr><td colspan="4">Scan repetition</td><td colspan="4" rowspan="2">One single scan, analysis using HAW setting = 0.020</td></tr>
<tr><td colspan="4">Analysis of data using automated setting and setting of HAW = 0.020</td></tr>
</table>

Table 2. Comparison of the housing conditions in facilities D and G

	Facility D	Facility G
Cage system	Conventional Type II cages with filter tops	Type II individually ventilated cages
Diet	Altromin 1314	Altromin 1314
Changing regimen	Once per week	Once per week
Mice per cage	Maximum 5	Maximum 5
Temperature (facility)	22°C ± 2	22°C ± 2
Humidity (facility)	55°C ± 10	55°C ± 10
Light cycle	6 am to 6 pm light, 6 pm to 6 am dark	6 am to 6 pm light, 6 pm to 6 am dark

Table 3. Statistical analysis* of the influence of housing conditions.

	Value	Std. Error	DF	p-value
Intercept	22.776	0.596	286	< 0.001
Age	0.085	0.003	286	< 0.001
Facility	-5.342	0.536	76	< 0.001
Sex	6.179	0.527	76	< 0.001
Strain	-7.549	0.527	76	< 0.001

*Linear mixed-effects model fit by REML for parameter weight. The used dataset includes all measurements of both facilities until the age of 300 days.

Table 4. Correlation among bone mineral content (BMC), bone mineral density (BMD), fat mass and lean mass data from two independent DEXA scans using two different modes of analysis*.

	Correlation coefficient of scan 1 vs. scan 2 using an automated analysis	Correlation coefficient of scan 1 vs. scan 2 using setting HAW=0.020
BMC	0.842 [0.789, 0.883]	**0.932 [0.908, 0.950]**
BMD	0.700 [0.609, 0.773]	**0.816 [0.755, 0.863]**
Fat mass	**0.922 [0.860, 0.936]**	0.903[0.864, 0.932]
Lean mass	**0.872 [0.827, 0.905]**	0.840 [0.786, 0.881]

*Confidence intervals are indicated in brackets.

Figure legends

Figure 1a. Weight curves over the entire experimental period for C3HeB/FeJ mice in facility D and facility G (by sex and strain).

Figure 1b. Weight curves over the entire experimental period for C57BL/6J mice in facility D and facility G (by sex and strain).

Figure 2. Development of fat mass (a), lean mass (b), BMC (c) and BMD (d) over time for male and female C3HeB/FeJ and C57BL/6J mice in facility G.

Figure 3. Fat mass (a), lean mass (b), BMC (c) and BMD (d) from male and female C3HeB/FeJ and C57BL/6J mice plotted against body weight.

Figure 4. Scatterplot matrix for BMD, BMC, lean mass, fat mass, soft tissue, and body weight data. The dashed line shows the regression line of a linear model. The solid line shows an estimated LOWESS smoothing line using locally-weighted polynomial regression (see Becker et al. 1988).

Figure 5a. Plot of data from first DEXA scan vs. data from the second scan using the automated analysis setting.

Figure 5b. Plot of data from first DEXA scan vs. data from second scan using a fixed HAW value of 0.020.