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## The Use of Different Statistical Metrics to Detect Physiological Changes in Metal-Overloaded Avian Bone

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### ABSTRACT

Slag from nickel smelting operations in the Sudbury basin in Ontario, Canada, has become ubiquitous. This material rich in heavy metals - particularly iron - upon ingestion has the potential to effect changes in physiology. In previous work, we analyzed the effects of dietary slag ingestion on several quantitative parameters of the tibio-tarsal bones in pigeons, including density, cortical thickness, bone mineral density, calcium and iron concentrations, Young's Moduli, and breaking strength. In the present work, we compute the Pearson correlation coefficients for all possible sets of bivariate correlations of the measured parameters for a control group fed a normal diet, and an experimental group fed a slag-based diet. Furthermore, the Pearson distance, which is a metric associated with the degree of "clustering" between two independent sets of bivariate correlations, was calculated for all corresponding pairs of bivariate correlations. A new metric - the Distance correlation R - is a measure of multivariate correlation which tests for any type of correlation, including linear correlation (or anticorrelation). In analogy to the Pearson distance, we introduce in this work the Distance correlation  $d_R$ , and a criterion for its statistical significance. On the existing data set, the Distance correlation R was also calculated for all 42 bivariate correlations, and the associated 21 values of  $d_R$  were computed. In the control group, the Pearson correlation revealed an anticorrelation between cortical thickness and breaking strength. In the experimental group, the metric revealed a correlation between breaking strength and bone mineral density, and an anticorrelations between: i) Young's Moduli and iron concentration, and ii) between cortical thickness and bone mineral density. Significant Pearson distances between: i) cortical thickness vs. breaking strength, ii) cortical thickness vs. bone mineral density, iii) density vs. bone mineral density, and iv) breaking strength vs. bone mineral density, were indicative of fundamental changes in bone physiology in the experimental group. The Pearson distance metric was thus effective in detecting physiological changes associated with the presence of metal overload in bone. The Distance correlation R revealed a significant correlation between cortical thickness and breaking strength in the control group, and between the calcium and iron concentrations in the experimental group. The  $d_R$  metric revealed significant loss in correlation between calcium concentration and bone mineral density in the experimental group. In summary, the statistical metrics used - and introduced in this work - are efficacious in detecting physiological changes in metal-overloaded bone.

### 1. Introduction

In statistics, the Pearson correlation coefficient  $r$  is a measure of the linear correlation between two variables; it has a value between -1 and +1, where 1 implies a complete positive linear correlation, 0 implies no linear correlation, and -1 implies a complete negative linear correlation. It was introduced by Karl Pearson [1]. Pearson's correlation coefficient between variables  $x$  and  $y$  for a population is defined as the covariance of the two variables divided by the product of their standard deviations. In equation form:

$$r = \frac{cov(x,y)}{\sigma_x \sigma_y} \tag{1}$$

where  $cov(x,y)$  is the covariance of  $x$  and  $y$ , and  $\sigma_x$  and  $\sigma_y$  are the population standard deviations of  $x$  and  $y$  respectively. The interpretation of a Pearson correlation coefficient depends on context; for example, a correlation of 0.70 may be very low if one is verifying a physical law using high-quality instruments, but may be regarded as very high in the biological sciences where there may be greater "noise" from complicating factors [2]. A distance metric associated with the degree of "clustering" between two independent sets of bivariate correlations for specific applications in the biological sciences, has been proposed by Fulekar [3]. This distance, called the Pearson distance  $d_{x,y}$ , is defined as,

$$d_{x,y} = |r_{x1,y1} - r_{x2,y2}| \tag{2}$$

where  $r_{x1,y1}$  and  $r_{x2,y2}$  are the Pearson correlations for the first and second data set respectively. Note that,

$$0 \leq d_{x,y} \leq 2. \tag{3}$$

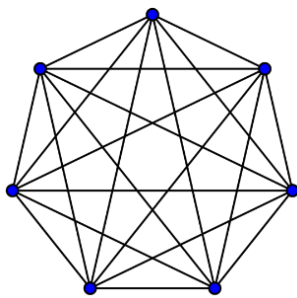
In the Fulekar interpretation,  $d_{x,y}$  is a measure of how tightly clustered the data for the two sets of bivariate correlations are, in other words the closer the value gets to 2, the less "clustered" the data, which is suggestive of fundamental differences in the processes under analysis.

In previous work, we examined mechanical, chemical, structural, and radiological changes in pigeon bone (*Columba Livia Domestica*), associated with the dietary intake of nickel recovery slag, which contains approximately 40% iron by weight [4,5]. The specimens were maintained on a seed diet and divided into a control group provided (normal) clean limestone grit, and an experimental group provided slag-based grit - both for a period of one year - after which their tibio-tarsal bones were harvested for analysis. Quantitative analytical methods included conventional density measurements (CD), caliper-based cortical thickness measurements (Th), bone mineral density measurements using Dual Energy X-ray Absorptiometry (BMD), calcium and iron concentration measurements using mass spectrometry (Ca) and (Fe), and the determination of Young's Moduli (YM) and breaking strength (BS), both in compression, using a universal testing machine. A Welch's t-test (single tail) was used to compare means of the seven quantitative parameters between control and experimental samples; in six of these parameters, a

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statistically significant difference was found ( $p \leq 0.05$ ) [6,7]. Extensive and complementary studies on the effects of slag ingestion on other organs and systems in these same specimens were undertaken by another group of investigators [8].

In this work, we examine the Pearson correlation coefficients for all possible sets of bivariate correlations of the measured parameters in our previous work, for both groups of specimens, experimental and control. It must be pointed out that this type of analysis only tests for a linear association in the bivariate, and establishes nothing regarding other types of possible associations such as quadratic or higher order, exponential, or sinusoidal correlations, etc. In our case, since we have seven parameters to correlate, the set of all possible pairings of measured parameters correspond to the edges of the  $K_7$  Complete Graph, with the vertices corresponding on a one to one basis with each of the seven measured parameters. A complete graph is a simple undirected graph in which every pair of distinct vertices is connected by a unique edge.  $K_n$  has  $n(n-1)/2$  edges (a triangular number), and is a regular graph of degree  $n-1$ , which can most conveniently be represented graphically (and symmetrically) as a regular  $n$ -polygon [9,10].  $K_7$  has 21 vertices (correlations). A graphical representation of  $K_7$  as a regular heptagon is presented in Fig. 1. Since we have two such data sets (control and experimental groups respectively), this results in a total of 42 possible correlations.



**Fig. 1** Graphical representation of  $K_7$  as a regular heptagon. In our work, each vertex represents a measured physical parameter, and the 21 non-directed edges represent a bivariate correlation

More recently, in a pair of landmark papers, Szekely and collaborators have introduced the Distance correlation  $R$  as a measure of multivariate correlation, which was further developed by Nott et al., and Li et al. [11–14]. The method is extremely general in that it is applicable to pairs of bivariate correlations with unequal dimensions, depending only on the Euclidean pairwise distance between data points. If the two variables are sampled from a bivariate normal distribution, the Distance correlation behaves very much like Pearson's correlation coefficient. However, because only Euclidean pairwise distances are taken into consideration, the method may be applied to inherently unobservable variables with only Euclidean pairwise distances entering the picture. Since it is a normalized distance between two vectors, the Distance correlation satisfies  $0 \leq R \leq 1$ , with  $R = 0$  only if  $X$  and  $Y$  are independent. It therefore tests for any type of correlation including linear correlation (or anticorrelation), as opposed to the Pearson correlation which tests exclusively for a linear correlation. The first report of the application of this new correlation metric in the biomedical sciences was published by Kong and collaborators [15]. These authors investigated the familial correlations in lifestyle factors and mortality, as part of a much larger, ongoing population-based study of age-related ocular disorders [16].

Analogous to the Pearson distance  $d_{xy}$ , we propose the concept of the Distance correlation distance  $d_R$  such that:

$$d_R = |R_1 - R_2| \quad (4)$$

where  $R_1$  and  $R_2$  are the Distance correlations for the first and second data set respectively. Note that

$$0 \leq d_R \leq 1. \quad (5)$$

Since this is a metric we are proposing for the first time, no accepted standard for statistical "significance" exists. We therefore propose (somewhat arbitrarily), a condition of  $d_R \geq 0.20$  for statistical "significance".

## 2. Experimental Methods

The methodology used to obtain the physical measurements used as raw data for this work, as well as the biological aspects of specimen handling and care, have been extensively described and we repeat here <https://doi.org/10.30799/jespr.192.20060102>

only as a brief of summary [4,8]. Previously, we examined changes in pigeon bone associated with the dietary intake of nickel mining slag, which resulted in a condition of metal (mostly iron) overload in the specimens. These were divided into a control group provided a normal diet and an experimental group provided the slag-based, metal-rich diet. Their tibio-tarsal bones were harvested for analysis. Quantitative analytical methods included conventional density measurements (CD), caliper-based cortical thickness measurements (Th), bone mineral density measurements (BMD) using dual energy x-ray absorptiometry, calcium and iron concentration measurements (Ca) and (Fe) using mass spectrometry, and the determination of Young's Moduli (YM) and breaking strength (BS). As selected examples of some of the experiments carried out for this work, we present in Fig. 2a a typical bone specimen being compressed in a 500 kN hydraulic Universal Testing Machine (Tinius Olsen Model 2000 SL UTM, Horsham, PA, USA). This machine consists of a hydraulic piston which compresses the sample at a carefully controlled rate, recording sample compression and applied force, up to the point of failure. Based on sample geometry, stress-strain data are generated and Young's Modulus is extracted by a "best-fit of the slope" algorithm. The machine automatically produces a stress-strain curve, a best fit value of (YM) (in metric units of MPa), and a (BS) value (in metric units of N). The specimen is stabilized in the upright position by inserting it in a plastic foam base. In Fig. 2b, a photograph of the sample specimen after failure is presented.

In this work, the Pearson correlation coefficient  $r$  was calculated for all 42 bivariate sets. Furthermore, the Pearson distance  $d_{xy}$  was calculated for all corresponding pairs of bivariate correlations, between the control and experimental groups, resulting in 21 values of  $d_{xy}$ . Similarly, on the same data set, the Distance correlation  $R$  was calculated for all 42 bivariate sets, and the corresponding 21 values of  $d_R$  were computed.

The Pearson correlations were calculated using the statistics package in Microsoft Excel 2010®. The Distance correlations were calculated using a purpose-written code in Python, as no known commercially available code for the Distance correlation exists. All physically measured parameters were given as a mean  $\pm$  standard error. All parameters were subjected to a single tail Welch's t-test - which allows for unequal sample sizes and variances - to determine statistical significance in differences in means between the two groups. A  $p \leq 0.05$  threshold was selected to indicate statistical significance, as indicated by ANOVA.



**Fig. 2** a) Bone specimen being compressed in a 500 kN hydraulic Universal Testing Machine (on left). The machine determines the Young's Modulus (YM) and breaking strength (BS) for the specimen. The specimen is stabilized in the upright position by inserting it in a plastic foam base. b) a photograph of the sample specimen after failure (on right)

## 3. Results and Discussion

There is no commonly accepted standard for statistical "significance" for the value of  $r$  in biomedical applications, and in fact different authors propose different standards. For example, Cohen proposed a minimum value of 0.70 for a "high" degree of correlation, whereas Fulekar argued that values as low 0.65 could be regarded as indicative of "high" correlation in complex biological systems [2,3]. In the absence of an accepted standard, we choose for this work a conservative value for  $r \geq 0.70$  as indicative of a "high" degree of correlation in a bivariate. By symmetrical argument,  $r \leq -0.70$  is considered in this work as indicative of a high degree of negative, or anticorrelation.

**Table 1** Pearson correlation coefficients " $r$ " for all bivariate correlations in the control data set

$r$	Fe	Ca	YM	BS	CD	BMD
Th	-0.616	0.7623	-0.4604	-0.8998	-0.4086	0.7131
BMD	-0.2393	0.2117	0.2511	-0.137	-0.7895	-
CD	0.0929	0.4202	-0.0699	-0.0004	-	-
BS	0.4316	-0.3955	0.7431	-	-	-
YM	-0.0403	-0.0136	-	-	-	-
Ca	0.38	-	-	-	-	-

In Table 1, the  $r$  values for all pairs of bivariate correlations in the control group are presented. In the "high" degree of correlation category we have the

calcium concentration (Ca) vs. cortical bone thickness (Th), with a value 0.7623. This is consistent with previous reports which associate high calcium concentrations in bone with thicker bones, in humans. For example, Matkovic and collaborators evaluated the effects of calcium supplementation on bone accretion and thickness in the transition from childhood into early adulthood. This study found significant increases in the cortical radius of the experimental subjects [17].

The next pair that presents a “high” correlation is breaking strength (BS) vs. Young’s Modulus (YM) with a value of 0.7431. The correlation can be understood in terms of the fact that both (YM) and (BS) are different ways of quantifying the mechanical “hardness” or “stiffness” of a solid body; thus, one can expect that an increase in one should correlate with a general increase in the other. This result is compatible with results reported by Kim and collaborators, who found that these two parameters are highly correlated in avian tibio-tarsal bones [18].

A “high” correlation was found between cortical bone thickness (Th) and bone mineral density (BMD), with  $r = 0.7131$ . Since (BMD) is a measure of the area density of bony tissue expressed in  $\text{g}/\text{cm}^2$ , it is to be expected that thicker bone would yield higher (BMD) readings, hence the high correlation. Positive correlations between (Th) and (BMD) have been reported in the literature. For example, Tingart and collaborators reported an  $r$  value of 0.84 between cortical humeral thickness and (BMD) measurements in humans and in a similar study, Lacroix and collaborators also reported a value of 0.84 [19,20].

An unusually “high” - and apparently counterintuitive - anticorrelation ( $r = -0.8998$ ) was observed between cortical thickness (Th) and breaking strength (BS). Based on elemental considerations, one would typically expect that a thicker bone would exhibit a greater breaking strength. However, at least one report was found in the literature in which such an anticorrelation was reported. Based on histological analysis and computer simulations, Silva and Gibson reported that in humans, an increase in vertebral trabecular bone thickness resulted in a reduction of breaking strength of up 73%. The authors hypothesized that the quality of the added bone was not only inferior to the original matrix, but actually had a frank detrimental effect on its overall mechanical properties [21]. Consistent with this hypothesis is our finding of a very strong anticorrelation ( $r = -0.7895$ ) between conventional physical density (CD) and bone mineral density (BMD). This anticorrelation can be understood if one again considers that (BMD) is an area density, such that if additional thickness is added to bone, this area density necessarily increases as the x-ray beam must traverse a greater path length. However, as conjectured by Silva and Gibson, the net effect of adding additional layers of bone, has a detrimental effect on the bone’s overall mechanical properties, including its conventional (physical) density [21].

In Table 2, the  $r$  values for all pairs of bivariate correlations in the experimental group (specimens with metal/iron overload in their diets) are presented. In the “high” degree of correlation category we have the bone mineral density (BMD) vs. breaking strength (BS) with  $r = 0.8732$ . These results are consistent with results published by other investigators. Conrad et al. and Hester et al. reported an increased correlation between (BMD) and (BS) in the femora of poultry subjected to iron overload conditions [22,23]. Furthermore, Kim and collaborators have reported that in humans, iron overload (elevated serum ferritin levels), is associated with an increased correlation between (BMD) levels and the risk of pathological fracture in the femur, a surrogate of breaking strength [24].

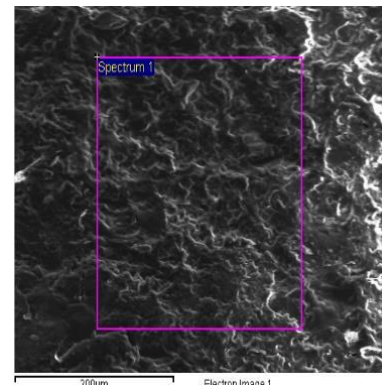
**Table 2** Pearson correlation coefficients “ $r$ ” for all bivariate correlations in the experimental data set

$r$	Fe	Ca	YM	BS	CD	BMD
Th	-0.353	0.0791	0.2929	0.3988	-0.3877	-0.9018
BMD	-0.0206	0.1801	0.0611	0.8732	0.8497	-
CD	-0.262	0.106	-0.1327	0.0218	-	-
BS	-0.5464	-0.3141	0.5286	-	-	-
YM	-0.7875	-0.6715	-	-	-	-
Ca	0.58	-	-	-	-	-

Also, in the experimental group, a “high” correlation was found between the conventional (physical) density (CD) and the (BMD), with  $r = 0.8497$ . This is in sharp contrast to an anticorrelation found between these two parameters for the control group ( $r = -0.7895$ ). It is clear that metal/iron overload has a significant effect on the correlation between these parameters. A strong positive correlation between (CD) and (BMD) under conditions of metal overloading has been reported in the literature. For example, Tsay et al. reported that in murine models under iron overload conditions, in-vivo (BMD) measurements are a better indicator of osteoporosis (and hence CD) than in non-overloaded conditions [25]. De Vernejoul et al. compared (BMD) and (CD) measurements in normal vs. iron-overloaded porcines, and concluded that the two parameters

correlate “significantly better” under conditions of iron overload [26]. Cork and collaborators argued in two different reports, that the strong correlation between (BMD) and (CD) in avian species under iron overload conditions (hemosiderosis), was associated with the high content of iron in bones [27,28]. Iron, being much denser than calcium (7.87 vs. 1.55  $\text{g}/\text{cm}^3$ ), increases the correlation between the two parameters.

A strong anticorrelation was also found between the Young’s Modulus (YM) and the iron concentration (Fe) with an  $r = -0.7875$ . It is clear from this anticorrelation that the iron overload in bone can have a detrimental effect on the mechanical properties of bone. In our previous work, we reported the existence of large cavities visible under electron microscopy examination, as seen in Fig. 3 (JEOL model 6400 Scanning Electron Microscope, JEOL Corp., Tokyo, Japan) [4]. These cavities are consistent with resorptive cavities lined with osteoclasts, and are indicative of cortical discontinuities, foci of woven bone formation, and/or spicules of fibroplasia, all consistent with previously reported histological findings [8]. Ward et al. suggested that these changes are thought to play a significant detrimental role in the physiology and mechanical properties of bones in iron-overloaded avian species [29]. Brissot and collaborators described generally similar changes in bony structure and performance in humans subjected to iron-overload conditions [30].



**Fig. 3** Electron micrograph of experimental (i.e. metal/iron overloaded) sample. Note the existence of resorptive cavities lined with osteoclasts, indicative of cortical discontinuities, foci of woven bone formation, and/or spicules of fibroplasia. Image acquisition parameters were: accelerating voltage = 30 kV, and beam current =  $1.0 \times 10^{-11}$  A

Lastly, an unusually “high” anticorrelation ( $r = -0.9018$ ) was found between cortical bone thickness (Th) and (BMD) in the experimental group. This value represents the highest magnitude for the value of  $r$  that we have found in our work. This finding is consistent with Silva and Gibson’s suggestion that the net effect of adding additional layers of bone, has a detrimental effect on the bone’s overall mechanical properties, including its BMD. However, it must be noted that the authors did not test their hypothesis in iron-overloaded specimens [21]. Orban, and Dorrestein et al., reported generally comparable anticorrelations between (Th) and (BMD) in iron-overloaded hens, and myna birds, respectively [31,32]. This anticorrelation is also consistent with our previous work, in which we reported the existence of large cavities visible under electron microscopy (Fig. 3) [4].

As mentioned previously, a distance metric called the Pearson distance  $d_{xy}$ , quantifies the degree of “clustering” between two independent sets of bivariate correlations, for specific applications in the biological sciences [3]. It is a measure of how tightly clustered the data for the two sets of bivariate correlations are; in other words the larger the value, the less “clustered” the data, which is suggestive of fundamental differences in the processes under analysis. We computed  $d_{xy}$  for all corresponding pairs of bivariate correlations between the control and experimental groups (21 pairs in total). These results are presented in Table 3. Fulekar has proposed that for biological applications, due to the complexity of the systems involved, a  $d_{xy} \geq 1.0$  should be considered indicative of two fundamentally different - or substantially modified - physiological processes [3]. We adopt this recommendation in our analysis.

**Table 3** Pearson distances  $d_{xy}$  between all control and experimental bivariate correlations

$d_{xy}$	Fe	Ca	YM	BS	CD	BMD
Th	0.263	0.6832	0.7533	1.2986	0.0209	1.6149
BMD	0.2187	0.0316	0.19	1.0102	1.6392	-
CD	0.3549	0.3142	0.0628	0.0222	-	-
BS	0.978	0.0814	0.2145	-	-	-
YM	0.7472	0.6579	-	-	-	-
Ca	0.58	-	-	-	-	-

The first pair of bivariate correlations to satisfy the recommended criteria, is the breaking strength (BS) vs. cortical thickness (Th) pair, with  $d_{xy} = 1.2986$ . This is due to the very strong anticorrelation ( $r = -0.8998$ ) in the control group bivariate correlation - consistent with Silva's suggestion that the net effect of adding additional layers of bone has a detrimental effect on its mechanical properties - and the rather weak correlation ( $r = 0.3988$ ) observed in the experimental group bivariate correlation [21]. In the case of the weak but positive correlation for the experimental group, this can be understood in terms of the excess amount of iron in the metal-overloaded specimens taking precedence over the detrimental effects of additional bone thickness, in determining breaking strength in compression. Young's Modulus is a measure of the stiffness of a solid, and correlates well with breaking strength. If one considers that Young's Modulus for steel, which contains at least 50% iron, is  $2 \times 10^{11}$  Pa, compared to a value of  $1.5 \times 10^{10}$  Pa for cortical femur (in humans), then a higher breaking strength is to be expected in metal/iron-overloaded specimens, and a significantly better correlation between (Th) and (BS) should follow [33].

Consistent with this last Pearson distance, is the Pearson distance of  $d_{xy} = 1.6392$  between the control and experimental group bivariate correlations of conventional physical density (CD) vs. (BMD). This distance is due to the very strong anticorrelation ( $r = -0.7895$ ) in the control group bivariate correlation and the strong positive correlation ( $r = 0.8497$ ) observed in the experimental group bivariate correlation. As explained by Frimeth et al., the (BMD) method is exquisitely sensitive to the presence of any high atomic number elements, especially heavy metals [34]. Furthermore, Pietrobelli and collaborators communicated results in which iron-overloaded humans presented elevated (BMD) indices, compared to normal control subjects [35]. Because the (BMD) method is exquisitely sensitive to the presence of heavy metals such as iron, and because conventional physical density (CD) is also obviously proportional to the amount of heavy metals present, one should expect a much better correlation between (BMD) and (CD) in the metal-overloaded specimens. This Pearson distance is a reflection of the (BMD) method's ability to detect the added presence of metals in the experimental specimens.

Along similar lines, we observed a  $d_{xy} = 1.0102$  between the control and experimental group bivariate correlations of breaking strength (BS) vs. (BMD). This distance is due to a rather weak anticorrelation ( $r = -0.1370$ ) in the control group bivariate correlation and the strong correlation ( $r = 0.8732$ ) observed in the experimental group bivariate correlation. The weak anticorrelation between (BS) and (BMD) is unexpected, given that (BMD) is considered a good indicator of fracture probability [17,20]. However, at least one report exists in the literature indicating that an anticorrelation between these two parameters is possible; Cheng and Coon reported precisely such an anticorrelation in work with hens [36]. On the other hand, a strong correlation between (BMD) and (BS) in iron-overloaded specimens has been previously reported. Conrad et al., and Hester et al. reported an increased correlation between (BMD) and (BS) in the femora of poultry subjected to iron overload conditions [22,23]. This same strong correlation has also been reported in humans by Kim and collaborators [24]. We can conclude that in this case, the Pearson distance method is effective in detecting the difference between normal and metal-overloaded specimens.

The last instance of a significant Pearson distance occurred between the control and experimental bivariate correlations of cortical thickness (Th) vs. (BMD), with a  $d_{xy} = 1.6149$ . This distance is due to the very strong correlation ( $r = 0.7131$ ) in the control group bivariate correlation and the very strong anticorrelation ( $r = -0.9018$ ) observed in the experimental group bivariate correlation. This last anticorrelation is the largest we encountered in our work. In the case of this pair of bivariate correlations, the strong correlation that existed in the control group bivariate correlation, was shifted to a strong (actually the strongest) anticorrelation in the experimental group. Furthermore, this was the only instance of a significant Pearson distance in which a correlation in the control group shifted to an anticorrelation in the experimental group. A strong correlation is to be expected in the control group since (BMD) is a measure of area density and thicker normal bone should be associated with increased area density. Tingart et al. and Lacroix et al. reported  $r$  values of 0.84 and 0.81 respectively, for this bivariate correlation in normal specimens [19,20]. Regarding the very strong anticorrelation between these two parameters in the experimental group (metal-overloaded), this is consistent with our previous work, in which we reported the existence of large cavities visible under electron microscopy (Fig. 3) and Silva's proposition that adding layers of bone thickness in metal-overloaded specimens results in physiological degradation [4,21]. Orban, and Dorrestein et al., also reported similar anticorrelations in iron-overloaded poultry specimens [31,32]. On a related note, Priest has suggested that contamination with heavy metals - specifically iron - results in the loss of the (BMD) method as an assessment tool for bone thickness, i.e. the correlation shifts from positive to negative [37]. In the case of this pair of bivariate correlations, the Pearson distance method suggests that

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fundamental physiological alterations are caused by metal/iron-overloading of the specimens.

Next, we turn our attention to the Distance correlation  $R$ . In their original paper, Szekely and collaborators proposed that the condition for statistical significance in Distance correlation be such that  $R \geq 0.95$ , which Kong et al. adopted for biomedical applications [11,15]. Without further justification, we adopt this standard in our work. In Table 4, the Distance correlation  $R_C$  values for all pairs of bivariate correlations in the control group are presented. We see that only one pair, cortical thickness (Th) versus breaking strength (BS) satisfies the Szekely-Kong criteria for statistical "significance". This significant correlation, which is a reflection of the strong negative or anticorrelation detected for this bivariate correlation by the Pearson method ( $r = -0.8998$ ), can be understood in terms of the mechanical characteristics of healthy bone. In healthy bone, the greater the cortical load-bearing thickness, the greater the expected mechanical strength of the bone under axial compression. This result is an example of the ability of the Distance correlation metric to detect any type of correlation, including a negative linear correlation (or anticorrelation).

**Table 4** Distance correlation coefficients  $R_C$  for all bivariate correlations in the control data set

$R_C$	Fe	Ca	YM	BS	CD	BMD
Th	0.83	0.92	0.82	0.95	0.88	0.86
BMD	0.66	0.9	0.93	0.86	0.88	-
CD	0.73	0.83	0.85	0.85	-	-
BS	0.76	0.85	0.94	-	-	-
YM	0.71	0.89	-	-	-	-
Ca	0.84	-	-	-	-	-

In Table 5, the Distance correlation  $R_E$  values for all pairs of bivariate correlations in the experimental group are presented. Only one pair, calcium concentration (Ca) versus iron concentration (Fe) satisfies the Szekely-Kong criteria for statistical "significance". This result suggests that iron uptake in bone is associated with the amount of calcium present, however since the Pearson correlation for this same bivariate correlation is not statistically significant (Table 2), we can rule out a simple linear correlation between the two variables. The correlation is therefore nonlinear, and of an inverse type. This constitutes an example of the Distance correlation method being able to detect a non-linear correlation, for which the Pearson method is insensitive.

**Table 5** Distance correlation coefficients  $R_E$  for all bivariate correlations in the experimental data set

$R_E$	Fe	Ca	YM	BS	CD	BMD
Th	0.85	0.75	0.93	0.89	0.86	0.94
BMD	0.69	0.68	0.74	0.94	0.9	-
CD	0.77	0.71	0.72	0.76	-	-
BS	0.9	0.84	0.94	-	-	-
YM	0.88	0.85	-	-	-	-
Ca	0.95	-	-	-	-	-

Finally, Distance correlation distances  $d_R$ , for all corresponding pairs of bivariate correlations between the control and experimental groups (21 pairs in total) were computed. These results are presented in Table 6. Since this is a metric we are proposing for the first time, no accepted standard for statistical "significance" exists. We therefore propose somewhat arbitrarily, a condition of  $d_R \geq 0.20$  for "significance". Only one bivariate correlation pair, bone mineral density (BMD) vs. calcium concentration (Ca) satisfies this criterion of "significance" for the distance of Distance correlation. This occurs because  $R$  drops from 0.90 for the control group bivariate correlation, to 0.68 for the same bivariate correlation in the experimental group. A strong correlation is expected in the control group for this bivariate correlation, given the well documented association between calcium content and (BMD) in both human, and avian bone [38,39]. For the experimental group in which dietary iron is absorbed in bone at the expense of calcium, a loss of the association between calcium content and (BMD) is to be expected. However, no reports of this loss of association have been found in the literature.

**Table 6** Distance correlation distance  $d_R$  between all control and experimental bivariate correlations

$d_R$	Fe	Ca	YM	BS	CD	BMD
Th	0.02	0.17	0.11	0.06	0.02	0.08
BMD	0.03	0.22	0.19	0.08	0.02	-
CD	0.04	0.12	0.13	0.09	-	-
BS	0.14	0.01	0	-	-	-
YM	0.17	0.04	-	-	-	-
Ca	0.11	-	-	-	-	-

#### 4. Conclusion

The Pearson correlation metric  $r$  revealed an unexpected anticorrelation between cortical thickness (Th) and breaking strength (BS) in the control group, given that elemental considerations would suggest that a thicker bone should exhibit a greater breaking strength. In the experimental group, the metric revealed a strong correlation between breaking strength (BS) and bone mineral density (BMD), as well as strong anticorrelations between Young's Modulus (YM) and iron concentration (Fe), and between (Th) and (BMD). None of these correlations (or anticorrelations) were observed in the control group. The Pearson distance  $d_{xy}$ , which is a metric associated with the degree of "clustering" between two independent sets of bivariate correlations, detected significant changes between the control and experimental groups for the following four pairs of bivariate correlations: (Th) vs. (BS), conventional density (CD) vs. (BMD), (Th) vs. (BMD), and (BS) vs. (BMD). These Pearson distances are indicative of fundamental changes in bone physiology, and we conclude that the Pearson distance metric is effective in detecting the presence of metal overload in bone. It must be emphasized that the Pearson correlation, and therefore the Pearson distance - by mathematical design - can only detect linear correlations. Therefore, the absence of a Pearson correlation, or of a "significant" Pearson distance, does not exclude the existence of a complex nonlinear correlation between the data in a bivariate.

The much more recently introduced Distance correlation  $R$ , which tests for all types of correlations, i.e. linear and/or non-linear, revealed a significant correlation between (Th) and (BS) in the control group. In this case, the Distance correlation metric is correctly confirming the strong negative linear correlation detected by the Pearson method. In the experimental group, the Distance correlation metric revealed a significant correlation between the calcium and iron concentrations (Ca) and (Fe). Since the Pearson metric produced no significant correlation for this bivariate, we conclude that the correlation between these two parameters in the experimental group must be nonlinear, and of an inverse type. Finally, the Distance correlation distance metric  $d_R$  which we introduce in this work, reveals a reduction or loss in the (Ca) vs. (BMD) correlation in the experimental group, compared to the control group. No such loss was detected by the Pearson distance metric, which can be interpreted as meaning that the correlation between (Ca) and (BMD) is nonlinear and possibly complex.

In summary, the statistical metrics used - and introduced in this work - are efficacious in detecting physiological changes in metal-overloaded bone.

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