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Areas With High Concentrations of Selenium in the Soil and Forage Produce Beef With Enhanced Concentrations of Selenium

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Abstract

Selenium (Se) is an essential trace element that provides many health benefits, including cancer prevention, improved immune function, and enhanced psychological function. Beef provides a significant portion of human dietary Se, and it is possible that modest portions of beef produced in areas with high-Se soil and forage could provide almost the entire Recommended Dietary Allowance (RDA). The present study has addressed the environmental conditions that result in the production of high-Se beef. One hundred and thirty-eight cull cows were obtained from twenty-one different ranches throughout the state of North Dakota. Ranches were chosen from five distinct geographic regions that, based on soil parent material, reports of Se deficiency, and previous soil and forage Se surveys, were likely to have high or

low Se concentrations in the soil. Grass and soil samples were taken during the growing season, and cattle were shipped to a commercial abattoir where skeletal muscle, diaphragm muscle, and liver samples were obtained. Hair and whole blood samples were taken one day prior to shipping. Selenium concentrations of all samples were determined by hydride generation atomic absorption spectroscopy. Geographic origin affected Se content of all samples ($p < 0.05$). Selenium concentrations in soil ($r = 0.53$; $p < 0.01$) and grass ($r = 0.63$; $p < 0.01$) were correlated to Se content of skeletal muscle. Selenium concentrations in whole blood, diaphragm, hair, and liver also were significantly correlated to Se content of skeletal muscle ($p < 0.01$). Cows that received Se in mineral supplements did not have significantly higher concentrations of Se in sampled tissues ($p > 0.05$). Results of this study suggest that the greatest source of variation in Se content of bovine skeletal muscle is the geographic region where the beef originates, and not production or management practices. Results also suggest that a 100g serving of high-Se beef could provide 100% of the RDA for Se.

Key Words: Selenium, Beef, Soil

Introduction

Selenium (Se) was demonstrated to be nutritionally essential in 1957 (Schwartz and Foltz, 1957). Selenium deficiency results in fatal disease conditions in humans and animals (Levander, 1986). Selenium functions in the active site of selenoproteins, and also is involved in immune and neuro-psychological function. A Recommended Dietary Allowance (RDA) of 55 g/d for women and 70 g/d for men has been determined based on the saturation of glutathione peroxidase (GSH-Px) enzyme activity (National Research Council, 1989).

Recent evidence indicates that consumption of Se in excess of the RDA may provide substantial cancer-protective benefits for humans. In a long-term, double-blind study (Clark et al., 1996), 200 g of supplemental Se supplied as high-Se yeast resulted in dramatic reductions in lung, colorectal, and prostate cancer. Cancer incidences have been demonstrated to be inversely related to geographic areas with high concentrations of Se in forage crops (Clark et al., 1991; Shamberger and Frost, 1969), and the incidence of prostate cancer has been reported to be inversely proportional to the prediagnostic Se content of toenail clippings (Yoshizawa, et al. 1998).

Publication of the cancer-protective benefits of Se has resulted in many people seeking to increase their Se intakes, but there are relatively few choices available for accomplishing this. Tablets of Se as selenite or high-Se yeast are available, but guidelines from the American Dietetic Association encourage people to consume nutrients through food whenever possible. However, the Se concentration of a particular food may be variable and dependent on the geographic origin of the raw agricultural product (Finley, 1996).

On average, beef is the single largest source of Se in the North American diet and provides almost 20% of total dietary Se (Shi and Spallholz, 1994; Holden et al., 1991). Similar to other foods, the Se concentration of beef depends on its geographic origin (Hoffman et al., 1972; Finley et al., 1996). Because Se from beef comprises such a large portion of total dietary Se, and because the Se content of beef is variable, the geographic origin of beef may greatly influence total dietary Se intake. Ninety-seven percent of ranchers from seleniferous areas in western South Dakota and eastern Wyoming reported consuming beef raised on their ranches; the Se intake of these ranchers was reported to be 54% greater than the American average (Longnecker et al., 1991).

A study in Canada found large differences in the concentrations of Se in beef, depending on the geographic origin of the animal (Hoffman et al., 1972). The Western Plains of the Dakotas are underlaid by a geographic formation that contains high concentrations of Se (Rosenfeld and Beath, 1964). This geological formation results in high concentrations of Se in the soil of parts of North and South Dakota, and consequently, the beef raised in this region may contain high concentrations of Se. The demonstrated health benefits of Se, the importance of beef in Se nutriture, and the potential enrichment of Se in the beef of animals raised on the high-Se soils of the western Dakotas suggest that consuming beef raised in these areas may be an ideal way of increasing dietary Se intakes. Consequently, the overall objectives of this study were to determine: a) the variation in concentrations of Se in beef produced in areas of North Dakota known to have high or low soil Se, b) the profile of Se in soil and forage from these areas and the relationship between Se in the environment and Se in the food chain, and c) which easily obtainable animal tissues could be used to predict the Se content of skeletal muscle.

Experimental Design

Twenty-one ranches in five distinct geographic regions throughout North Dakota participated in this study. Regions overlying geologic formations known to produce forages either high or low in Se were chosen as target areas. The ranches were located in western Bowman and Slope Counties (southwest ND; SW), Sioux County (south-central ND; SC), Williams County (northwest ND; NW), Morton and southern Oliver Counties (central ND; C), and the sandhill region of Richland and Ransom Counties (southeast ND; SE). The SW and SC regions were chosen because the parent soil material is Cretaceous aged shales, primarily Pierre Shale (Clayton et al., 1980) that is associated with high Se soils (Rosenfeld and Beath, 1964). The NW sampling area was selected because soil and forage Se surveys in the late 1940s showed it was a high Se area (Byers et al., 1948). The C and SE regions were chosen because of scattered reports of Se deficiency and the lack of seleniferous geologic material suggested they may be low Se regions (Clayton et al., 1980). Producers were contacted in each region and recruited on a voluntary basis.

Grass and Soil Sampling

Grass and soil samples were taken from the final pasture of the grazing rotation. Pastures were divided into 2 - 6 similar sized quadrats, and five grass and five soil samples (each soil sample was a composite of 10 subsamples) were taken/quadrat. A 1-meter clipping square was randomly placed to collect and separate grass, standing dead grass and broadleaf plants. A soil probe was used to collect soil samples from 0 to 25 cm in depth. Feed labels from mineral supplements were examined to determine if Se sources other than forages were present in the diet.

Tissue Sampling

Carcass and organ samples were collected from one hundred and thirty-eight cull cattle that were shipped from the participating ranches to a commercial abattoir. Hair (from the tail) and blood (jugular venipuncture into EDTA) samples were collected one day prior to slaughter. Carcass and organ samples (50-150 g of liver, skeletal muscle, and diaphragm muscle) were collected at the time of slaughter. All samples were frozen at -30^{o} C until analysis.

Selenium Analyses

Liver, skeletal muscle, and diaphragm samples were lyophilized, and ground into a powder prior to analysis. Hair samples were cleaned with acetone and distilled-deionized water using the method of van Ryssen et al. (1994). Forage and soil samples were oven dried at 60^{o} C for 72 hours and ground through a 2 mm screen. Soil samples were also homogenized in a soil roller mill prior to analysis. Tissue, hair, and grass samples (0.3-0.5 g) were digested in concentrated nitric acid (J.T. Baker, Inc., Phillipsburg, NJ) by a previously described procedure (Finley et al., 1996). Selenium was determined by hydride-generation atomic absorption spectroscopy (HGAAS).

Soil was analyzed for soluble Se by a modification of the procedure described by Black et al. (1965). A suspension of approximately 5 g of ground, homogenized soil was refluxed in 30 ml of deionized distilled water for 1 hour, centrifuged and filtered. Concentrated hydrochloric acid was added to the filtrate, and samples were heated to 60 C.

Quality control was maintained by analysis of triplicate standards of bovine liver (NIST # 1577b) or apple leaves (NIST # 1515 for forage analysis; U.S. Department of Commerce National Institute of Standards and Technology, Gaithersburg, MD 20899) with each batch run of samples. Runs were acceptable if the NIST analyzed values fell within the stated range. The run-to-run coefficient of variation averaged 1.3%. The within-run coefficient of variation averaged 1.1%. The Se content of blank samples averaged 0.14 ng/ml. The detection limit for Se analyzed by this method was 0.001 ng/ml. All samples were analyzed in duplicate.

Statistical Analysis

Data were analyzed as a nested design with ranch within region. The experimental unit was individual animal for tissue samples, and ranch for forage and soil samples. Data were analyzed by the GLM procedure of SAS (SAS, 1990) and Tukey's studentized range test was used to separate means. Pearson correlation coefficients were computed to determine inter-sample relationships. T-tests were used to compare means of animals exposed to Se supplements and animals without Se supplements, and to compare Se concentration means between skeletal and diaphragm muscle (Steel and Torrie, 1980). Regression equations were determined with the REG procedure of SAS using the best R^2 option (SAS, 1990). Selenium content of hair was influenced by hair color; consequently, hair analyses were blocked on hair color.

Results

Selenium concentration in animal samples

Muscle, blood, diaphragm and hair samples collected from animals from the NW region contained the greatest concentrations of Se (Table 1) whereas animal tissue and organ samples from the SE region always contained the least Se. The rank order of Se concentrations in muscle, blood, diaphragm and hair was NW > SC > SW > C > SE, and for liver it was SC > NW > C > SW > SE. The Se concentration of forage (Table 2) also was highest in the NW and lowest in the SE regions; the rank order was NW > SC > SW > C >SE. The NW region had the highest concentration of soluble Se in the soil but the rank order was not similar to that of Se in animal tissues or forage.

Associations between carcass Se concentration and Se concentrations of other organs and tissues.

Se concentrations in skeletal muscle (round) from the carcass were most strongly associated with Se concentrations of whole blood (r = 0.66, p = 0.0001, Figure 1a) diaphragm (r = 0.65, p = 0.0001, Figure 1b) and grass (r = 0.63, p = 0.0017, Figure 1c). Associations between muscle Se concentrations and Se concentrations in the liver (r = 0.36, p = 0.0001 Figure 1d), hair (r = 0.51, p = 0.0001, Figure 1e) and soil (r = 0.53, p < 0.01, Figure 1f) were significant but not as strong.

Supplemental Se did not increase the concentration of Se in any tissue or organ (p > 0.05), but the association between Se in liver and skeletal muscle of cattle not exposed to Se supplements was much stronger (r = 0.50,) than for supplemented animals (r = 0.23). Age of animal was not significantly associated with Se concentration of skeletal muscle (p > 0.05). Dark hair had a significantly higher Se concentration (1.47 g Se/g) than light hair (1.04 g Se/g; p < 0.0001, Table 4), and there was a stronger association between Se concentration in light hair and muscle (r = 0.65) than between Se concentration in dark hair and muscle (r = 0.46).

When all variables were included in a linear regression model, the best predictor of Se in skeletal muscle was [-0.022 + 0.60(whole blood Se) + 0.567(diaphragm Se) + 0.094 (soluble soil Se)], (R^2 = 0.80). When only non-invasively collected variables were considered, the best predictor was [-0.0086 + 0.98(whole blood Se) + 0.057(hair Se) + 0.094(soluble soil Se)], (R^2 = 0.75).

Discussion

Numerous animal and human studies have demonstrated the health benefits of Se. Selenium supplementation of an extremely deficient human population in the People's Republic of China prevents the occurrence of potentially fatal Keshan Disease (Chen et al. 1981). Intakes of Se in excess of the RDA may improve neuro-psychological function (Finley & Penland 1998). Recent evidence suggests that adequate Se status is necessary for optimal immune function (Beck et al. 1994). These reports, and the anti-cancer benefits of Se reported by Clark et al. (1996), have created much interest in increasing Se intakes, although if one wishes to obtain it through the diet there are few rich sources of Se. The present study demonstrates that beef from high-Se areas could be used to greatly increase dietary Se, and identifying such high-Se beef also may be a means of improving the often negative image of beef as a food.

Per capita consumption of red meat has declined substantially in the past few decades. Although the reasons for the decline are complex, certainly a primary reason is the popular misconception of red meat as a food with only negative health effects, i.e., it is a primary contributor to heart disease and major cancers. And because of the negative publicity, the health benefits of red meat are often ignored. Perhaps one of the most positive nutritional aspects of beef is the amount of trace elements, including Se, that it contributes to the diet. Meat enriched in Se may provide an opportunity for positive marketing and perhaps an opportunity to develop a specialty nutrient-enhanced product.

The present study clearly demonstrates that the concentration of Se in edible beef is consistently higher when animals are raised in areas where the underlying geologic features are known to be high in Se. The area of the state that did not have high-Se geological features (SE

region) consistently produced animals with the lowest tissue Se concentrations and areas with geologic features known to be high in Se consistently produced cattle with the highest tissue Se concentrations. The geographic origin of the animals was a more important determinant of the Se concentration of beef than the presence or absence of supplemental Se. Geographic area resulted in Se concentrations of 0.27 to 0.67 g Se/g, whereas providing supplemental Se only increased mean Se concentrations from 0.41 g/g (no supplemental Se) to 0.46 g Se/g ($p = 0.07$). The animals used in this study were cull cows that had grazed the particular ranch for multiple years (average of 6.6 years old); consequently, differences were not a reflection of a single growing season, but were cumulative over many seasons. A human selenomethionine (SeMet) kinetic metabolism model developed by Swanson et al (1991) showed that SeMet has a slow whole body turnover rate (363 [illegible] 21 days) because of efficient recycling of SeMet. Extrapolating this to cattle consuming SeMet from seleniferous range suggests Se accretion might have a cumulative effect over time. Although age of the cow did not affect Se concentrations in any organ or tissue in the present study, the study lacked a diversity of animal ages and replications per ranch to properly test this hypothesis.

The potential significance of increasing the Se concentration of beef can be appreciated when its contribution to total dietary Se intake is calculated. Assuming a national average of approximately 0.2 g Se/g beef, and assuming an intake of 100 g of this beef/d, then an individual's Se intake from beef would be 20 g/d, or less than [illegible] the adult female and less than 1/3 the adult male RDA. Conversely, the beef of individual animals from NW North Dakota exceeded 1.0 g Se/g beef, and if a person consumed 100 g of such beef, their Se intake would be in excess of 100 g/d, or in excess of the RDA for either men or women.

Linear regression models were used to determine the best predictors of Se concentrations of skeletal muscle. The best predictors among samples that could be obtained in the field were Se concentration of whole blood, hair and soil Se solubles. Although the association between Se concentration of grass and Se concentration of skeletal muscle was stronger ($r = 0.63$) than the association between skeletal muscle Se and soluble soil Se ($r = 0.51$), the model included Se in soil, but not Se in grass as a predictor. (Soluble Se in soil was measured as opposed to total Se as it more accurately reflects Se available to plants (Olson and Moxon, 1939)). Based on this model, one could quickly identify potential areas that would produce high-Se beef carcases by sampling soil from the area and blood and hair from the animals. Another regression model was used to show that the concentration of Se in an individual carcass could be well predicted by measuring Se in whole blood and diaphragm; both are samples that could be easily obtained from a slaughter house and without damage or loss of value to the carcass. Although the liver is a major pool of Se in the body, the Se concentration of liver was not a good predictor of Se in skeletal muscle.

There are few reports of the association between whole blood Se and skeletal muscle Se of cattle grazing on pasture. Whole blood is considered to be indicative of long-term Se status (Ullrey, 1987; van Ryssen et al., 1989) because of the relatively long half-life of erythrocytes (Behne and Wolters, 1983; van Ryssen et al., 1989). Hair also can be obtained easily by non-invasive means, but the association between muscle-Se and hair-Se was not as strong ($r = 0.51$) as muscle-Se and whole blood-Se ($r = 0.66$). Using the Se concentration of hair to predict Se concentration of muscle may be complicated by hair color. In this study darker-colored hair was higher in Se than lighter-colored hair, this has also been noted in steers grazing native range (van Ryssen et al., 1994). The concentration of Se in both hair and muscle is affected by the chemical form of Se in the diet. van Ryssen et al. (1989) reported that the Se concentration of wool from sheep fed high-Se wheat was almost threefold higher than animals given the same amount of Se as selenite. Selenium fed to

rats as SeMet is associated with hemoglobin protein in blood, whereas Se from selenite is more likely to be incorporated into GSH-Px (Beilstein and Whanger, 1986). These effects of chemical form on Se deposition into tissues results in greater accumulation of Se in animals fed SeMet, as compared to animals fed other forms such as selenite (Beilstein and Whanger, 1988). Although the dietary form of Se consumed by cattle in this study was not determined, it is assumed that most Se in grass, like wheat, was in the form of SeMet (Olson et al., 1970).

Although the present study has demonstrated that the total Se concentration of beef may be increased by raising the animal in a high-Se area, it has not addressed the potential health benefits to humans of Se from beef. The health benefits of Se apparently depend in part on the chemical form of the Se consumed. Selenium salts are most effective for producing selenoproteins (Beilstein & Whanger 1988), and SeMet is most effective for increasing the body stores of Se. High-Se yeast used in the cancer study of Clark et al. (1996) is a mixture of many forms of Se including SeMet and methylated forms of Se, so it is not certain what is the active anti-cancer metabolite. Some suggest that mono-methylated Se is the active anti-cancer metabolite (Ip & Ganther 1990/); consequently, forms of Se most easily metabolized to this form may be the most efficacious against cancer. Monomethylated Se (MMSe) is formed by methylation of the reduced selenide. Selenomethionine may be converted to MMSe, after following the trans-selenization pathway but it is easier for it to directly substitute for methionine in proteins. The conversion of selenocysteine (SeCys) to MMSe is a relatively faster process.

Although most cancer studies indicate that SeMet is not as effective as other forms of Se for inhibiting carcinogenesis, the actual efficacy of a particular form of Se as a cancer protective agent may not be simple to predict. Brazil nuts have high concentrations of Se of which SeMet is assumed to be the primary form (Ip and Lisk, 1994a). Selenium from Brazil nuts has been demonstrated to be as effective, if not more effective, than Se from selenite in preventing DMBA-induced mammary tumors (Ip and Lisk, 1994b). Moreover, cancer protection is not the only biological function of Se, and SeMet is very effective in restoring tissue Se concentrations and thus, providing a long-term pool of Se for selenoprotein production (Shi and Spallholz, 1994). Consequently, the mixture of chemical forms of Se found in meat may provide multiple health benefits; further investigation is needed to elucidate these potential benefits.

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Table 1. The Se content of tissue and organ samples collected from cattle carcasses originating from five distinct geographical locales of North Dakota. Selenium concentration determined by HGAAS.[1,2,3]

	Region				
	NW	**SC**	**SW**	**C**	**SE**
Musc.	0.67 ± .04^{a}(23)	0.47 ± .03^{b}(20)	0.40 ± .02^{bc}(69)	0.38 ± .05^{b,c,d}(8)	0.27 ± .03^{d}(14)
Liver	0.68 ± .06^{a}(23)	0.76 ± .06^{a}(19)	0.49 ± .04^{b}(63)	0.61 ± .08^{a,b}(8)	0.47 ± .06^{b} (14)
Blood	0.49 ± .02^{a}(21)	0.36 ± .02^{b}(21)	0.35 ± .01^{b}(71)	0.29 ± .02^{c} (7)	0.27 ± .02^{c} (15)
Diaph.	0.54 ± .03^{a}(23)	0.42 ± .03^{b}(21)	0.38 ± .02^{c}(69)	0.35 ± .04^{c} (8)	0.26 ± .03^{d} (14)
Hair	1.78 ± .07^{a}(21)	1.51 ± .07^{b}(20)	1.17 ± .04^{c}(71)	1.01 ± .11^{c,d} (8)	0.72 ± .08^{d} (15)

[1] Values are means (mg/kg) ± SE; means with different superscripts in the same row are significantly different ($p < .05$).
[2] Se concentrations expressed on a wet weight basis.
[3] Number in parentheses is number of samples

Table 2. The Se content of dried forage and soil collected from the pastures of cull cattle. Pastures were on ranches scattered throughout five distinct geographical locales of North Dakota. Selenium concentration determined by HGAAS.[1,2,3]

	Region					
	NW	**SC**	**SW**	**C**	**SE**	**p value**
Grass	0.85 ± .08^{a}(4)	0.48 ± .07^{a,b}(4)	0.40 ± .05^{a,b}(9)	0.20 ± .13^{ab}(2)	0.17 ± .11^{b} (3)	0.02
Soil	0.84 ± .12^{a}(5)	0.07 ± .04^{b} (4)	0.14 ± .03^{b} (9)		0.39 ± .06^{a,b}(3)	0.04

[1] Values are means (mg/kg) ± SE; means with different superscripts in the same row are significantly different ($p < .05$).
[2] Se concentration of grass samples expressed on a dried weight basis.
[3] Number in parentheses is number of sampled ranches.

Table 3. Effect of Se supplementation on Se content of lyophilized, ground skeletal muscle; liver; diaphragm muscle, and whole blood taken from cull cows from five distinct geographic regions in North Dakota as determined by HGAAS [1]

Tissue	Se in Supplement	N	No Se in Supplement	N	p value
Skel. musc.	0.46 ± .02	58	0.41 ± .02	58	0.07
Liver	0.56 ± .03	58	0.56 ± .02	52	1.00
Whole Blood	0.36 ± .01	58	0.35 ± .01	59	0.40
Diaph. musc.	0.41 ± .01	60	0.38 ± .01	57	0.09

[1]Values are means (mg/kg) ± SE

Table 4. Effect of hair color on Se content of hair washed with acetone and distilled-deionized water, taken from the switch of the tail of cull cows from five distinct geographic regions of North Dakota as determined by HGAAS [1,2,3]

Hair color	N	Se content
Black	39	1.47 ± .06[a]
Brown	44	1.20 ± .05[b]
Blonde	59	1.04 ± .05[c]

[1] Values are means (mg/kg) ± SE; means with different superscripts are significantly different.
[2] Means blocked by region.
[3] p value < 0.001.

Figure 1. The association of Se concentrations in the skeletal muscle with Se concentrations in other organs, tissues, grass and soil. Cull cattle from different geographic areas of North Dakota were slaughtered in a commercial abattoir and organ and tissue samples were

collected. Grass ands soil samples were collected from the pasture that the animals were taken from. The Figure shows the association between Se concentrations in the carcass and Se concentrations in: a) whole blood; b) diaphragm; c) grass; d) liver when all animals were used; e) hair; f) soluble soil Se; g) liver from animals not exposed to supplemental Se.

Figure 1A

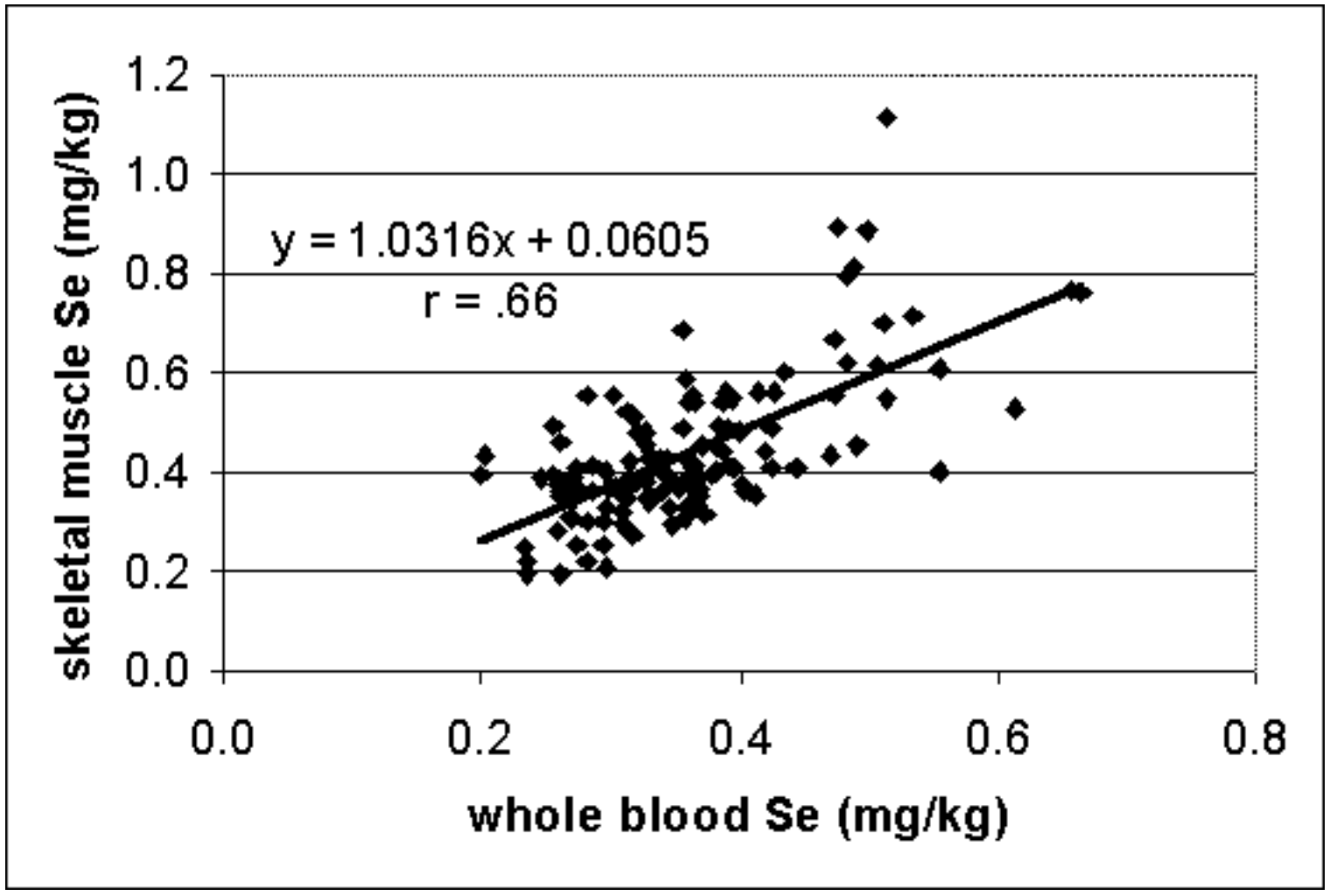


Figure 1B

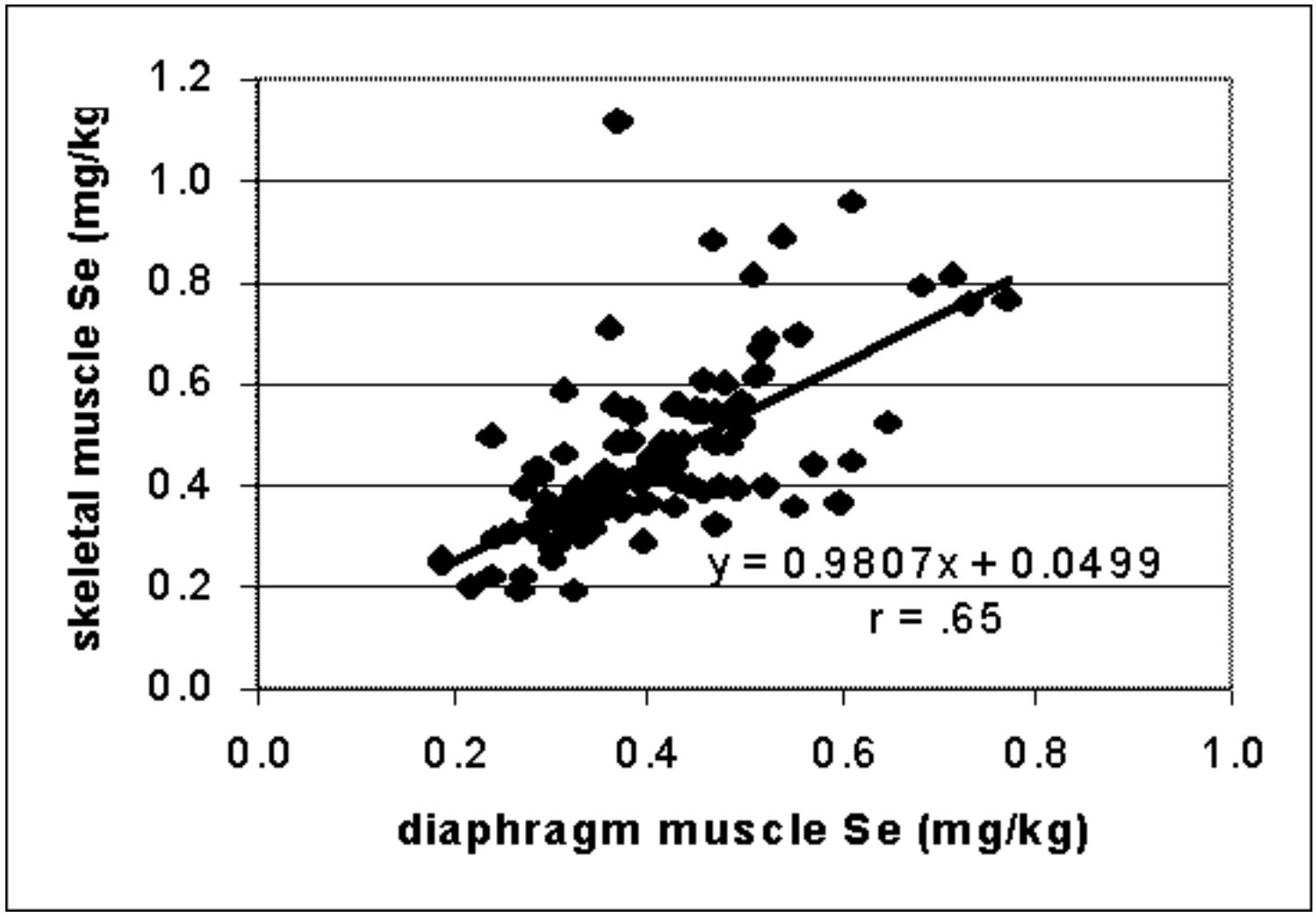


Figure 1C

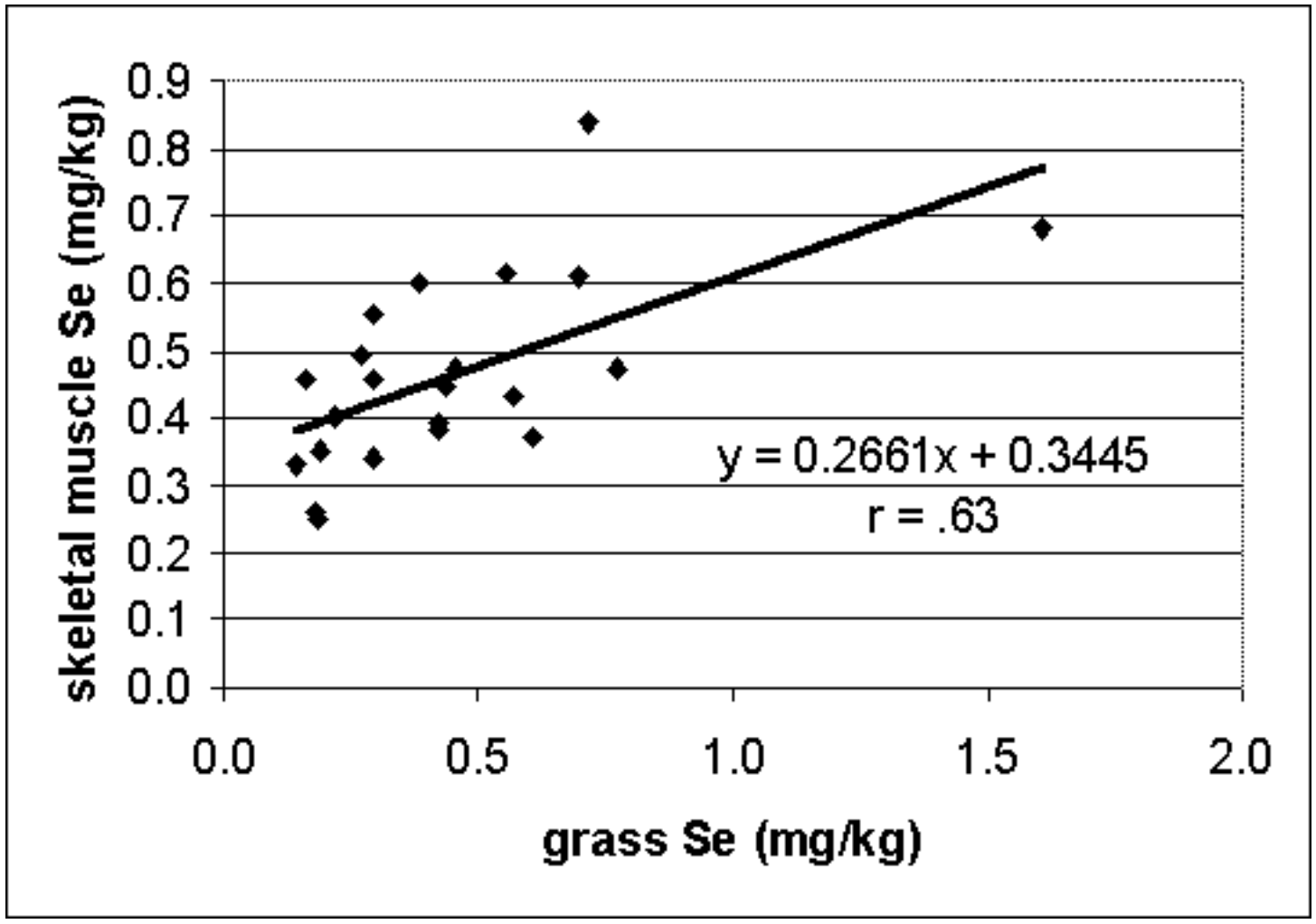


Figure 1D

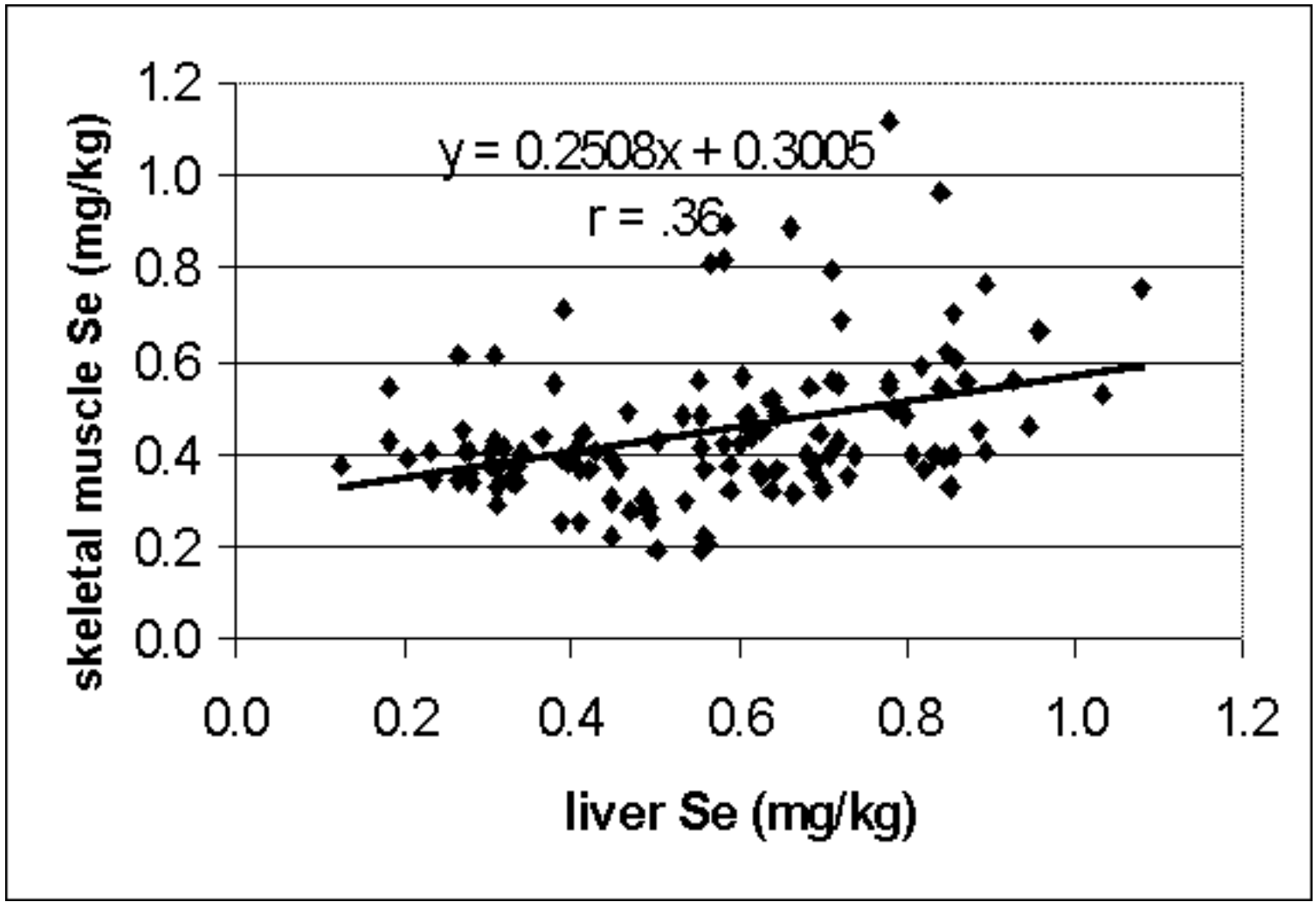


Figure 1E

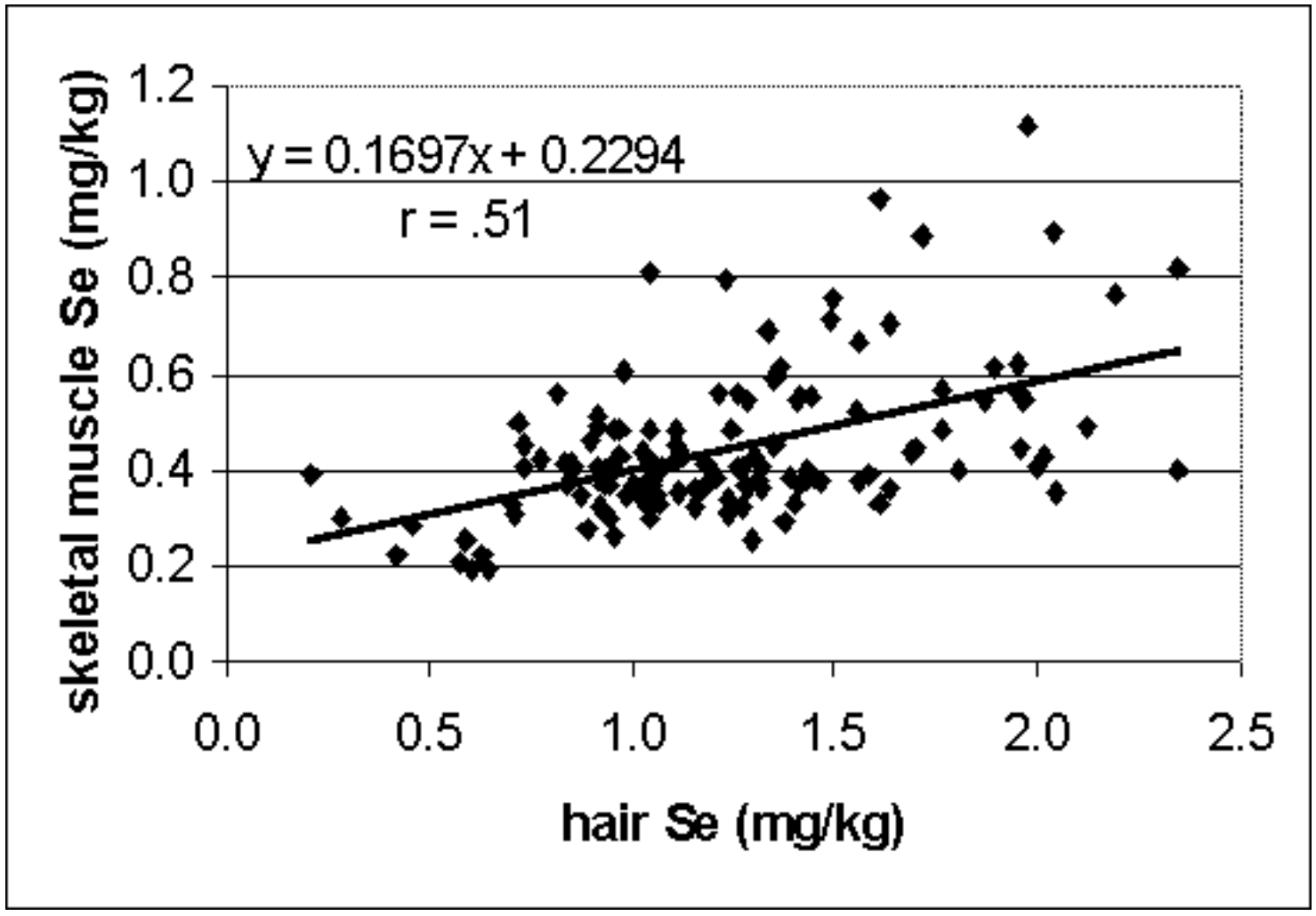


Figure 1F

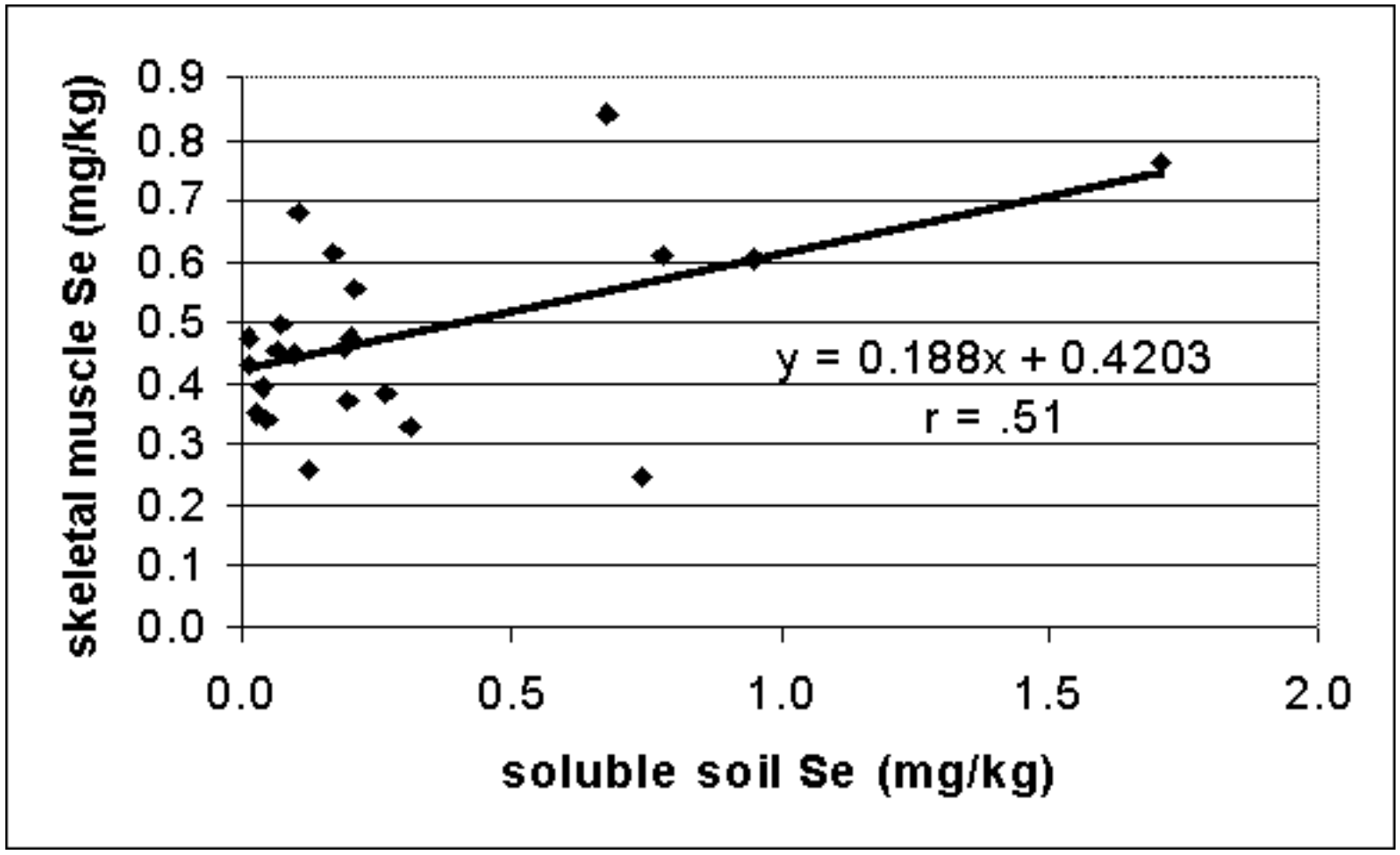
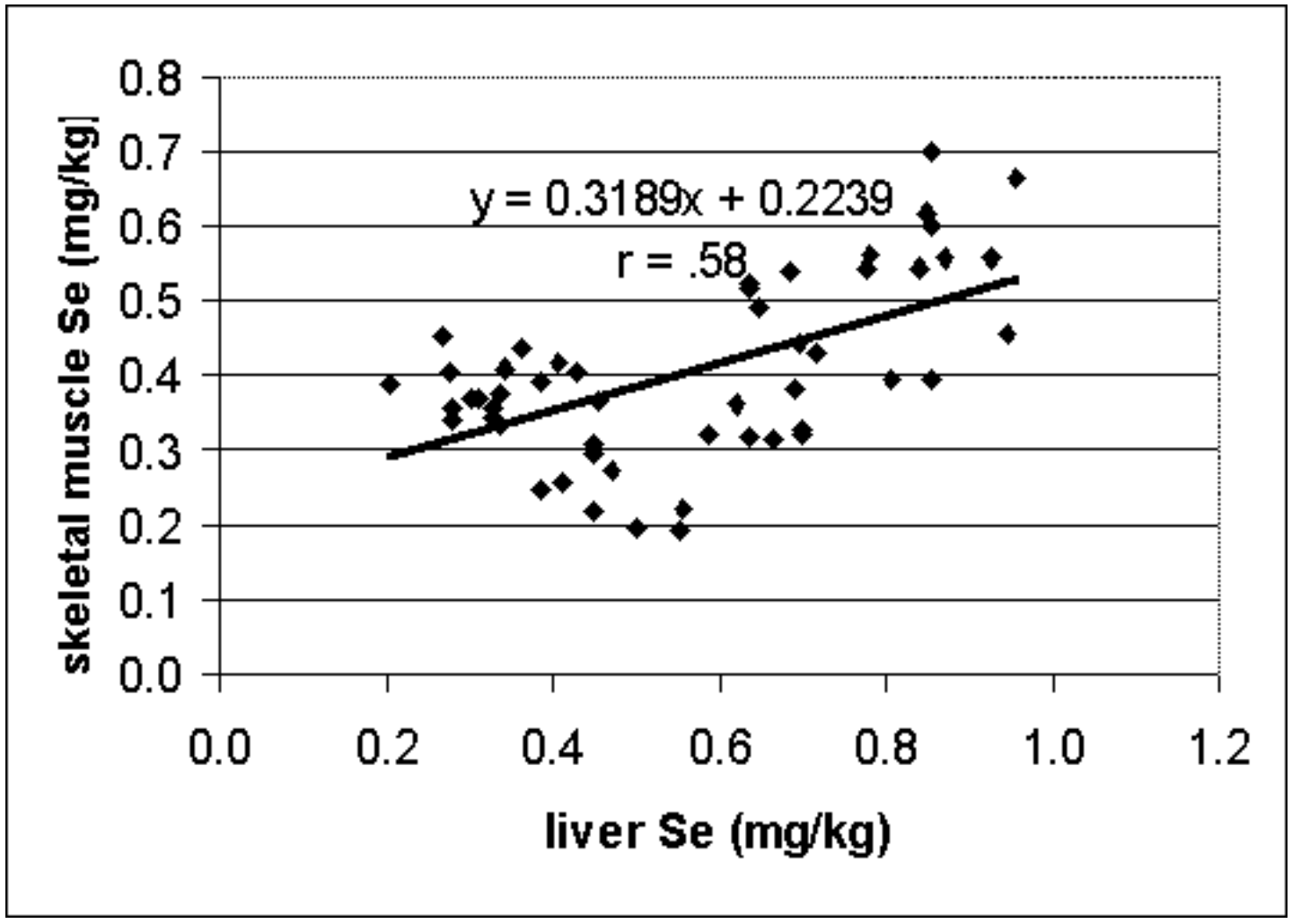


Figure 1G



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