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Meta-analysis of the relationship between collagen characteristics and meat tenderness

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Abstract

Meta-analysis methods were used to investigate the effects of collagen content, solubility and pyridinoline cross-link content on Warner-Bratzler shear force (WBSF) and sensory tenderness in major meat species. Data was collected from the literature on pork, beef and lamb and analysed independently for each species. The beef data was categorized into subgroups according to muscle (loin and other muscle) and age (young, <18 months and old, ≥18 months). The results showed that in beef, collagen content and pyridinoline cross-link content were positively correlated with WBSF and negatively with sensory tenderness, while collagen solubility was negatively correlated with WBSF. The correlation coefficients were greater in other beef muscles than loin. Significant correlations between collagen content and tenderness attributes were observed in old beef animals. However, non-significant relationships and large variations were found in pork and lamb. More studies with various muscles and ages are required for an in-depth understanding of the relationship between collagen characteristics and meat tenderness.

Key words: meta-analysis, collagen, tenderness, correlation, muscle, age

1. Introduction

Tenderness is one of the most important factors affecting consumers' perception of meat palatability. Tenderness is defined as "the resistance to shear or the toughness of meat" (Chandraratne et al., 2006). Several factors affect meat tenderness. Connective tissue is believed to define the "background toughness" of meat (Sentandreu et al., 2002). Connective tissue comprises collagen and elastin fibers embedded in a matrix of proteoglycans (Lepetit, 2008) with collagen being the most abundant protein in connective tissue. The concentration, properties and architecture of collagen play a vital role in the texture of raw meat (Nishimura et al., 1996). Denaturation and solubilization of collagen during cooking also affects the tenderness of cooked meat (Purslow, 2014).

Attempts have been made to correlate collagen characteristics with tenderness to quantify the effects of these characteristics on tenderness (Chambaz et al., 2003; Listrat et al., 2020). Collagen content and solubility have been the most commonly studied attributes. However, contradictory findings for the relationship between collagen characteristics and tenderness attributes exist in the literature. For example, collagen content was correlated with Warner-Bratzler shear force (WBSF) of raw meat (Nishimura et al., 2009; Torrescano et al., 2003), although the correlation coefficients (Pearson's r) varied between cooked beef, lamb and pork. The correlations between collagen content and instrumental or sensory tenderness were strong (Dransfield, 1977; Rhee et al., 2004), weak (Destefanis et al., 2000; Wheeler et al., 2000) or non-significant (Hopkins et al., 2013; Li et al., 2007). Similar results exist for the variability in the correlation between collagen solubility and tenderness attributes (Fang et al., 1999; Silva et al., 1999). In addition, the closeness of fit of these correlations varied among breeds, muscles and other factors (Serra et al., 2008; Starkey et al., 2016). Finally, variations exist in conditions and measurements among studies, such as age, sex and ageing time (Camacho et al., 2017; El Jabri et al., 2010; Girolami et al., 2009). A meta-analysis of published data offers one approach to reveal the general relationship between collagen characteristics and sensory and shear force attributes of meat.

Consequently, the objectives of the present meta-analyses were 1) to use statistical models to determine relationships between collagen content, collagen solubility, WBSF and sensory tenderness scores, and 2) to determine whether species, muscle and age affect these relationships.

2. Materials and methods

2.1 Study inclusion

A literature search was conducted in Web of Science using the search terms collagen, tenderness and meat (n=570), collagen, shear and meat (n=463) or pyridinoline, tenderness and meat (n=17) resulting in a combined total of 781 papers. From these papers, 73 peer-reviewed journal articles were selected which were published in English and reported tenderness attributes (peak WBSF and sensory tenderness score) and collagen characteristics (collagen content, solubility and pyridinoline cross-link content) of beef, pork and lamb and these were used to build generalized linear models. There were 12 publications for pork, 45 publications for beef and 16 publications for lamb that were eventually used for the meta-analyses. From each publication, breed, sex, age (months), ageing (days), muscle, cooking methods and cooking temperature (°C) were included as random factors. The means of collagen content, collagen solubility, WBSF and sensory tenderness scores from each paper were collated for subsequent statistical analysis. Publications that did not report the dispersion of data were excluded. In all selected publications, the collagen assays were conducted on raw meat and measurements of WBSF were on cooked meat. The unit of collagen content was mg/g raw meat. If hydroxyproline content was reported as a proxy for collagen content, a conversion factor of 7.25 was used for calculating collagen content (Starkey et al., 2015). Collagen content reported as mg/g dry matter was converted to a wet weight basis by multiplying by 30%, which is the percentage of dry matter in meat (Aaslyng et al., 2003; Yven et al., 2005). Collagen solubility was expressed as a percentage of total collagen. Pyridinoline cross-link content was calculated to the unit of mol/mol collagen, with the molar mass of collagen 3.0×10^5g/mol. The peak WBSF of cooked meat was standardized to units of N and data with a unit of kg was multiplied by 9.81 for conversion. Sensory tenderness scores were standardised, and converted where necessary, to a 10-point-scale with 1 being the toughest and 10 being the most tender.

Beef data were also subjected to additional meta-analysis of the correlation coefficients relating collagen characteristics and tenderness scores or WBSF. Another literature search was conducted in Web of Science using the search terms collagen, tenderness and beef (n=407) or collagen, shear and beef (n=325), resulting in a combined total of 461 papers. From these papers, 23 peer-reviewed journal articles were selected that were published in English and reported correlation coefficients (Pearson's r) between tenderness attributes (WBSF and sensory tenderness score) and collagen characteristics (collagen content and solubility) of beef. Pyridinoline cross-link content was excluded due to small number of suitable papers. Random factors for preliminary analysis were recorded as above. The correlation coefficients (Pearson's r) between collagen content and WBSF, collagen solubility and WBSF, collagen content and sensory tenderness score as well as collagen solubility and sensory tenderness were collated for subsequent statistical analyses. For all publications selected the number of samples (n) used for correlations were identified in the studies. Studies including correlations with raw meat WBSF were excluded.

2.2 Generalized linear model methodology

Generalized linear models of tenderness attributes and collagen characteristics were built by GenStat (16th edition, VSN International). Data from pork, beef and lamb were analyzed separately. For the beef data, different random factors were included in the models in preliminary analysis (table S3). As the inclusion of muscle or age in the model markedly affected the slope and its p-value for beef data, this data was divided into two different muscle groups, loin (*Longissimus thoracis* or *lumborum*) and other muscles (*Adductor, Biceps brachii, Biceps femoris, Deep digital flexor, Extensor carpi radialis, Flexor digitorum, Gastrocnemius, Gluteobiceps, Gluteus medius, Infraspinatus, Long digital extensor, Medial digital extensor, Peroneus tertius, Pectoralis profundus, Psoas major, Rectus abdominis, Rectus femoris, Semimembranosus, Semitendinosus, Supraspinatus, Serratus ventralis, Tensor fascia latae and Triceps brachii*), or two age groups. The two age groups were young animals less than 18 months and old animals aged 18 months or older. The two regression models used were as follow:

Model 1: $y = \beta_0 + \beta_1 \mathrm{Col} + \sum \beta_i R_i + \varepsilon$

Model 2: $y = \beta_0 + \beta_1 \mathrm{TCol} + \beta_2 \mathrm{ColS} + \sum \beta_i R_i + \varepsilon$

Where y is tenderness attributes (WBSF or sensory tenderness), Col is collagen characteristics (collagen content, solubility or pyridinoline cross-link content), β_1 is the regression slope of tenderness attributes on collagen characteristics, $\sum \beta_i R_i$ is the combined effects of references with a total number of i, and ε is the random error. In model 2, TCol represents collagen content and ColS represents collagen solubility.

Model 1 was used for all species and beef data with different subgroups. Models 2 was used for beef data. Both collagen content and solubility were independent variables in model 2. The slopes and R-squared values of the models were recorded. A generalized linear model of sensory tenderness on WBSF was also built (table S4).

2.3 Meta-analysis on correlations

Meta-analysis was conducted on the beef data using the correlation coefficient (r) as the effect size. Weightings were calculated using the number of measurements within each study. Analysis of the data was conducted using RStudio (RStudio, PBC) with the dmetar package (Harrer et al., 2019) using a random-effects model. The I^2 statistics were used to assess heterogeneity. Subgroup analyses were conducted using random-effects model for r between collagen content and WBSF and sensory tenderness and collagen solubility and WBSF. The two subgroups were loin and other muscles classified as in section 2.2. Forest plots were used to visualize the results. The overall effects, 95% confidence interval, prediction interval, heterogeneity (I^2) and p-value of the model were presented. Funnel plots were plotted to examine publication bias. A meta-regression of r across age (months) was also conducted using a mixed-effect model.

3. Results

3.1 Regression model of tenderness attributes on collagen characteristics

As shown in Table 1, for beef, collagen content contributed positively to WBSF (slope = 1.70±0.589, $p<0.01$) and negatively to sensory tenderness (slope = -0.21±0.067, $p<0.01$). Collagen solubility was negatively related to WBSF (slope = -0.49±0.151, $p<0.01$). No significant relationship was found between collagen solubility and sensory tenderness (Table 1). Pyridinoline cross-link content was positively related to WBSF (slope = 103.2±20.0, $p<0.001$) and negatively to sensory tenderness (slope = -3.99±2.01, $p=0.058$). In contrast, the correlations between collagen characteristics and sensory properties within pork and lamb were not significant and large variations were observed. Also, the number of articles for pork in particular, as well as for lamb, was limited. There was a negative relationship between WBSF and sensory tenderness in beef but not enough data for pork and lamb (table S4).

Beef data were further interrogated by being subjected to different regression models. After including muscle or age in the model, the slopes and their p-value of tenderness attributes on collagen characteristics changed more significantly than other factors (table S3). Therefore, beef data were divided into different muscle groups or age groups for further analysis.

More than half of the studies on beef included in the analyses utilized loin muscle. In the loin muscle, no significant relationship was found between collagen characteristics and tenderness attributes, although the trends were similar to those of all muscles (Table 2). In other muscles, significant relationships of WBSF and sensory tenderness on collagen content were observed ($p<0.05$). The slope of WBSF on collagen content was positive (slope = 1.88±0.865) and that of sensory tenderness on collagen content was negative (slope = -0.61±0.218). Pyridinoline cross-link content contributed positively to WBSF (slope = 98.2±21.1, $p<0.001$). No significant relationship was found for sensory tenderness. The R^2 of other muscles was lower than that of loin muscles in all models.

In young animals, the only significant relationship was between collagen content and sensory tenderness (slope = -0.301±0.106, $p<0.01$), but the R^2 of the model was only 46.8% (Table 3). In older animals, collagen content was positively related to WBSF (slope = 2.48±1.11, $p<0.05$) and negatively to sensory tenderness (slope = -0.515±0.115, $p<0.001$). Collagen solubility or pyridinoline cross-link content was not significantly related to tenderness attributes in either age group.

When both collagen content and solubility were utilized as independent variables, WBSF was positively related to collagen content (slope = 1.75±0.628, $p<0.01$) and negatively with collagen solubility (slope = -0.54±0.145, $p<0.001$) (Table 4). Collagen content contributed negatively to sensory tenderness (slope = -0.22±0.080, $p<0.05$), while collagen solubility contributed positively to sensory tenderness (slope = 0.082±0.0294, $p<0.05$).

3.2 Correlation coefficients between tenderness attributes and collagen characteristics

The estimated r between collagen content and WBSF was 0.23 ($p<0.001$) but the heterogeneity was high ($I^2 = 88\%$) (Fig. 1). Collagen solubility was negatively correlated with WBSF ($r = -0.16$, $p = 0.003$) with a moderate heterogeneity ($I^2 = 28\%$). For the correlation between collagen content and sensory tenderness, the estimated r was low ($r = -0.18$, $p = 0.001$) and I^2 was high (56%) (Fig. 2). The correlation between collagen solubility and sensory tenderness was not significant ($p = 0.47$). The funnel plots did not show marked asymmetry, indicating that there were no evidence of publication bias in all analysis (figure S2).

In the subgroup analysis with different beef muscle groups, there was no difference between loin and other muscle in the correlation between collagen content and WBSF (Fig. 1). However, the heterogeneity of loin was higher than that of other muscles ($I^2 = 96\%$ and 49%, respectively). Regarding the correlation between collagen content and sensory tenderness, heterogeneity was higher in loin ($I^2 = 52\%$) than in other muscles ($I^2 = 37\%$). In addition, the estimated r of loin muscle ($r = -0.05$, $p = 0.52$) was significantly lower than that of other muscles ($r = -0.27$, $p<0.001$) (Fig. 2). A similar result was observed in the correlation between collagen solubility and WBSF in which the r of loin muscle was -0.02 ($p = 0.82$), while that of other muscle was -0.22 ($p<0.001$) (Fig. 1). For the meta-regression of correlation coefficients across age, no significant effects of age could be determined (table S5).

4. Discussion

The major outcome of this study was that collagen content and solubility were significantly but weakly correlated with tenderness attributes in beef, with more significant correlations observed in muscles other than loin and in old animals. These findings were in agreement with the results presented by Chriki et al. (2013) who used metadata to study the contribution of collagen characteristics to beef tenderness.

The relationship between collagen characteristics and meat tenderness varied among species. Few studies compared collagen characteristics and their contribution to tenderness among species. Koohmaraie et al. (1991) found that beef loin showed a higher collagen content than pork and lamb, but lamb exhibited the highest WBSF. Wheeler et al. (2002) reported that the correlation between collagen content and sensory tenderness of loin was significant in beef but not in pork. Although the biological function of connective tissue in muscle is the same in all species, muscle composition, collagen characteristics, post-mortem proteolysis and tenderness of meat differs among species (Dransfield et al., 1981; Koohmaraie et al., 1991; Rødbotten et al., 2004). In addition, the relative maturity of cattle, pigs and sheep are different at slaughter, which affects connective tissue maturity and its contribution to meat toughness (Blanco et al., 2013; Koohmaraie et al., 1991). Therefore, the contribution of collagen characteristics to tenderness may differ between species. Consequently, the models built in the present study were applied separately for different species. In the present study, the only significant relationships were observed in beef. However, the numbers of articles and measurements were lower in pork and lamb than for the beef data. There were many variations between studies including breeds, age and sex, resulting in large standard error of the means. In addition, most studies of pork were on *Longissimus*. The correlations between collagen characteristics and tenderness attributes were weak in pork *Longissimus* (Gondret et al., 2006; Hovenier et al., 1993). More studies with various treatments such as breeds, age and muscles, applied to pork and lamb, are required.

For beef, both the regression method and the meta-analysis showed a positive correlation between collagen content and WBSF. Negative correlations were found between collagen content and sensory tenderness as well as between collagen solubility and WBSF. These findings agreed with previous studies with various muscles or breeds (Destefanis et al., 2000; Listrat et al., 2020; Rhee et al., 2004). However, in the present study, the Pearson's r of these correlations were low (-

0.30 < r <0.30), although they were significant. In a meta-analysis involving European beef, Chriki et al. (2013) also found weak correlations between collagen characteristics and tenderness attributes. Dransfield et al. (2003) reported that collagen content was only correlated with compression of raw meat but not cooked meat. Although collagen contributes to the toughness of raw meat, its properties change during cooking. Cooking temperature is critical to the effects of collagen on the tenderness of cooked meat (Christensen et al., 2000). In many previous studies, the meat samples were usually cooked to an internal temperature of 60 to 75°C (Bureš & Bartoň, 2012; Jurie et al., 2007; Sifre et al., 2005). The weak correlations between collagen characteristics and tenderness may be explained by the fact that the cooking of meat above 60°C results in an increase in WBSF, which is mainly attributed to the myofibrillar and sarcoplasmic proteins (Christensen et al., 2000). The contribution of collagen to meat toughness did not dominate at these cooking temperatures. Also, the temperature used to investigate collagen solubility in previous studies ranged from 70 to 90°C, which was higher than the cooking temperature normally used in sensory evaluation (Monsón et al., 2004; Silva et al., 1999). At cooking temperatures of 65-75°C, the measured solubilized collagen did not coincide with the expected amount, hence the effect on the tenderness of cooked meat was weak.

In addition to weak correlations, the heterogeneity of these models was moderate to high, indicating the large variations between studies, including rearing practice of animals, muscles, ageing time and cooking methods (Christensen et al., 2013; Palka, 2003; Serrano et al., 2007), all of which can influence collagen characteristics. In addition, the methods for measuring collagen characteristics and for sensory evaluation varied among studies. Finally, the precision of collagen content measurements was low in muscles with low collagen content (Etherington & Sims, 1981).

Although the correlations between collagen content and solubility and tenderness attributes were weak, the increase in the density of heat-stable collagen cross-links was positively related to increasing toughness, which was also shown by several studies (Listrat et al., 2020; Wang et al., 2016; Wu et al., 2021). However, McCormick (1999) reported that increased density of pyridinoline cross-link was related to increased collagen solubility and WBSF. Purslow (2014) proposed that there were two populations of collagen in which one fraction was easily degraded by ageing and cooking, while the other one was more resistant. Collagen solubility only measured the proportion of the insoluble fraction of collagen after cooking, but the mechanical strength and biochemical properties of the insoluble fraction determined the cooked meat toughness (Purslow, 2018). Therefore, although the slopes were significant when both collagen content and solubility were included in the models, their standard error of means were still high. Collagen content and the cross-link profile of the insoluble fraction of collagen might be better predictors for meat toughness (McCormick, 1999; Purslow, 2018). Therefore, both the quantity and quality of collagen contribute to meat toughness. Although both WBSF and sensory tenderness were found to be correlated with collagen characteristics, the relationship between WBSF and sensory tenderness varied in literature (Destefanis et al., 2000; Lorenzen et al., 2003). WBSF parameters did not reflect perceived tenderness by consumers well when comparing meat with different textural properties (Bouton et al., 1975; Warner et al., 2021). Consequently, peak WBSF is not a good predictor for connective tissue strength, which may explain the weak correlation between collagen characteristics and WBSF.

After including muscle in the beef models, the p-values of all slopes changed markedly. Collagen characteristics and muscle properties differed in different muscles, leading to extensive textural differences among muscles. Therefore, the contribution of collagen characteristics to tenderness varied among muscles. More than half of the measurements made were on loin muscles. No significant correlation between collagen characteristics and tenderness was found in the loin and its heterogeneity was high. The amount of connective tissue is low in the loin muscles compared to most of the other muscles (Rhee et al., 2004; Sifre et al., 2005). Other factors, such as sarcomere length, play a more critical role in determining the tenderness of loin muscles (Wheeler et al., 2000). Furthermore, the relatively low collagen content in loin muscles could induce a lower technical precision when measuring total and soluble collagen content, resulting in a wide range of variations in collagen characteristics among studies (Listrat & Hocquette, 2004). Collagen characteristics were not good predictors of tenderness in loin muscles.

In contrast, regression analysis showed significant relationship between collagen content and tenderness attributes as well as between pyridinoline cross-link content and WBSF in other muscles. Stronger correlations between collagen content and sensory tenderness and between collagen solubility and WBSF were observed in other muscles compared to the loin muscles (Fig. 1&2). Compared to loin muscles, some muscles, such as *Semimembranosus* (SM), *Semitendinosus* (ST) and *Triceps brachii* (TB), contain higher amounts of connective tissue (Jurie et al., 2007;

Sifre et al., 2005) and this may be attributed to the functional differences between muscles. To adapt to their role of myofascial force transmission and growth, the amount, composition and architecture of connective tissue varies between muscles (Purslow, 2010). The loin muscles are positional muscles, while SM, ST and TB are locomotive muscles. During physical activity, locomotive muscles require greater functional effort than positional muscles and thus have a higher collagen content (Dubost et al., 2013). Therefore, the contribution of collagen characteristics to tenderness was more significant in the locomotive muscles.

Despite the stronger correlations and lower I^2, the R^2 of regression models of other muscles was still low, perhaps because of the grouping together of the various muscles with differing physicochemical properties. Apart from their functional requirements, the type of muscle fibers also affects collagen content. Muscles with a higher amount of slow switch fibers have a greater collagen content (Kovanen et al., 1984; Nakamura et al., 2003). In addition, collagen solubility and the quantity, quality and maturity of cross-links differ between muscles. For example, *M. infraspinatus* has a relatively high collagen concentration, but its collagen solubility is also high, resulting in relatively tender meat (Keith et al., 1985; Modzelewska-Kapituła et al., 2016). This observation was in agreement with the regression model with both collagen content and solubility as independent variables. As collagen characteristics varied among muscles, it was better to investigate them individually. In the present study, there was not enough data for individual muscles other than the loin muscles. More studies on different muscles are required to determine the effect of muscle on the relationship between collagen characteristics and tenderness.

After including age in the beef regression model, the slope of collagen content changed slightly, while the slope of collagen solubility and pyridinoline cross-link content showed large changes. Thus, the effect of age on the relationship between tenderness and collagen was greater for collagen solubility and pyridinoline cross-links than for collagen content. As animals age, the collagen solubility decreases (Gerrard et al., 1987; Schönfeldt & Strydom, 2011) and this occurs in all muscles, breeds and sexes (Boccard et al., 1979; Schönfeldt & Strydom, 2011). The decline in collagen solubility is related to the conversion of heat-labile cross-links to heat-stable cross-links (McCormick, 1999; Shimokomaki et al., 1972). In the present study, the slope of the relationship between tenderness attributes and collagen solubility or pyridinoline cross-link content was not significant in either age group. In older animals, as their collagen solubility was low, the effect of collagen solubility on tenderness was less significant. Also, the pyridinoline cross-link content was based on total collagen content. As mentioned earlier, the cross-link profile of the insoluble population of collagen contributed to the toughness of cooked meat (Purslow, 2018). The changes in the quantity, quality and maturity of cross-links of the insoluble collagen may be a better explanation for the changes in contribution of collagen to meat tenderness with age. When age was considered as a continuous variable, no significant effect of age could be determined in the meta-regression analysis. This was likely because most of the studies collected used animals aged between 12 and 18 months. Thus, there were not enough data on older animals to see any trends.

The highest concentration of collagen in muscles occurs at birth and this gradually decreases until 6 to 8 months of age (Boccard et al., 1979; Nishimura et al., 1996). This is in contrast to contractile proteins as neonatal cattle need the strength to stand immediately after birth (Boccard et al., 1979). Hence connective tissue shows much earlier development than contractile proteins. Cross et al. (1984) found that beef collagen content increased from 6 to 12 months and decreased from 12 to 18 months, while Wang et al. (2016) reported that collagen content in beef barely changed from 18 months onward. Nevertheless, Schönfeldt and Strydom (2011) reported that collagen content was not correlated with age in 16 beef muscles. Other authors showed that the changes in collagen content with age differed among breeds, muscles and sex (Boccard et al., 1979; Gerrard et al., 1987). In the present study, significant relationships were found between collagen content and sensory tenderness in both age groups, and between collagen content and WBSF in old animals. A possible explanation for the two significant relationships in old animals was that collagen solubility was low in older animals. The insoluble portion of collagen in the old animals is so significant that collagen content, rather than collagen solubility, dominates (Young & Braggins, 1993). However, the correlation coefficient between collagen content and tenderness attributes did not change with age in our meta-regression analyses. More studies on the change in correlations between collagen content and tenderness attributes across age groups are required.

5. Conclusion

Collagen characteristics affected meat tenderness, but the relationship varied among species, muscle and age. The present meta-analyses showed that the relationship between WBSF and sensory tenderness, and collagen content and solubility of cooked meat, was significant but weak and was affected by muscle and age. Limited studies were available on pork and lamb as well as on muscles other than loin muscles. More studies on different species, muscles and age are needed to show their effects on the relationship between collagen characteristics and tenderness attributes. Individually, the contribution of collagen characteristics to tenderness was small, but together the quantity and quality of collagen were critical in determining meat tenderness. Apart from collagen content and solubility, the types of collagen, collagen network architecture, and especially the quantity, quality and maturity of collagen cross-links play important roles in meat texture. Further studies should include these collagen properties when investigating the relationship between connective tissue and tenderness.

Declarations of interest

None.

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Author contribution

Xiying Li: Conceptualization, Methodology, Data curation, Formal analysis, Writing-Original draft preparation. Minh Ha: Validation, Writing-Reviewing and Editing. Robyn Warner: Validation, Writing-Reviewing and Editing. Frank Dunshea: Conceptualization, Methodology, Writing- Reviewing and Editing.

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Table 1. Number of articles and measurements, regression coefficient (slope, mean ± standard error) and R-squared value of the regression model of tenderness attributes, Warner-Bratzer shear force (WBSF, N) and sensory tenderness (1 = tough to 10 = tender), on collagen characteristics (collagen content, mg/g fresh meat; collagen solubility %; pyridinoline cross-link content, mol/mol collagen) of different species

		Collagen content				Collagen solubility				Pyridinoline cross-link			
	Species	Number of articles	Number of measurements	Slope †	R^2 (%)	Number of articles	Number of measurements	Slope†	R^2 (%)	Number of articles	Number of measurements	Slope†	R^2 (%)
WBSF	Beef	29	112	1.70±0.589**	69.4	24	91	-0.49±0.151**	75.4	7	28	103.2±20.0***	68.5
	Pork	7	18	6.48±4.54	86.8	2	6	1.71±1.17	82.1	2	4	-40.0±198.0	90.2
	Lamb	14	54	0.34±0.781	55.7	7	23	-0.28±0.298	59.5	2	4	-26.0±196.0	‡
Sensory tenderness	Beef	21	69	-0.21±0.067**	49.6	14	44	0.0445±0.0281	51.1	9	35	-3.99±2.01	51.7
	Pork	6	21	-0.15±0.108	53.2	4	16	-0.0066±0.0180	93.5	1	2	§	§
	Lamb	5	14	0.26±0.940	‡	2	8	-0.26±0.216	‡	1	2	§	§

[†] Model: $y = \beta_0 + \beta_1 \text{Col} + \sum \beta_i R_i + \varepsilon$, where y is WBSF or sensory tenderness, Col is collagen content, solubility or pyridinoline cross-link content, and $\sum \beta_i R_i$ is the effect of references. [‡] Residual variance exceeded variance of response variate. **p<0.01, ***p<0.001. [§] Not enough data for analysis.

Table 2. Regression coefficient (slope, mean ± standard error), number of measurements (n) and R-squared of the regression model of tenderness attributes, Warner-Bratzer shear force (WBSF, N) and sensory tenderness (1 = tough to 10 = tender), on collagen characteristics, collagen content (mg/g fresh meat), collagen solubility (%) and pyridinoline cross-link content (mol/mol collagen), of different beef muscles

		Collagen content			Collagen solubility			Pyridinoline Cross-link		
	Muscle[†]	n	Slope[‡]	R^2 (%)	n	Slope[‡]	R^2 (%)	n	Slope[‡]	R^2 (%)
WBSF	Loin	65	1.76±1.53	76.6	56	-0.27±0.280	78.5	5	277.0±119.0	90.6
	Others	47	1.88±0.865*	54.4	35	-0.61±0.218*	63.2	16	98.2±21.1***	54.2
Sensory tenderness	Loin	30	-0.40±0.210	68.8	22	0.027±0.0351	82.1	13	2.03±9.59	37.7
	Others	39	-0.15±0.089	21.4	22	-0.072±0.0352	7.3	19	-3.78±2.08	39.6

[†] Loin = *Longissimus thoracis* or *lumborum*. Others = *Adductor, Biceps brachii, Biceps femoris, Deep digital flexor, Extensor carpi radialis, Flexor digitorum, Gastrocnemius, Gluteobiceps, Gluteus medius, Infraspinatus, Long digital extensor, Medial digital extensor, Peroneus tertius, Pectoralis profundus, Psoas major, Rectus abdominis, Rectus femoris, Semimembranosus, Semitendinosus, Supraspinatus, Serratus ventralis, Tensor fascia latae and Triceps brachii.* *$p<0.05$, ***$p<0.001$. [‡] Model: $y = \beta_0 + \beta_1 Col + \sum \beta_i R_i + \varepsilon$, where y is WBSF or sensory tenderness, Col is collagen content, solubility or pyridinoline cross-link content, and $\sum \beta_i R_i$ is the effect of references. R^2 is the percentage of variance explained by the model.

Table 3. Regression coefficient (slope, mean ± standard error), number of measurements (n) and R-squared of the regression model of tenderness attributes, Warner-Bratzer shear force (WBSF, N) and sensory tenderness (1 = tough to 10 = tender), on collagen characteristics, collagen content (mg/g fresh meat), collagen solubility (%) and pyridinoline cross-link content (mol/mol collagen), of beef with different age groups

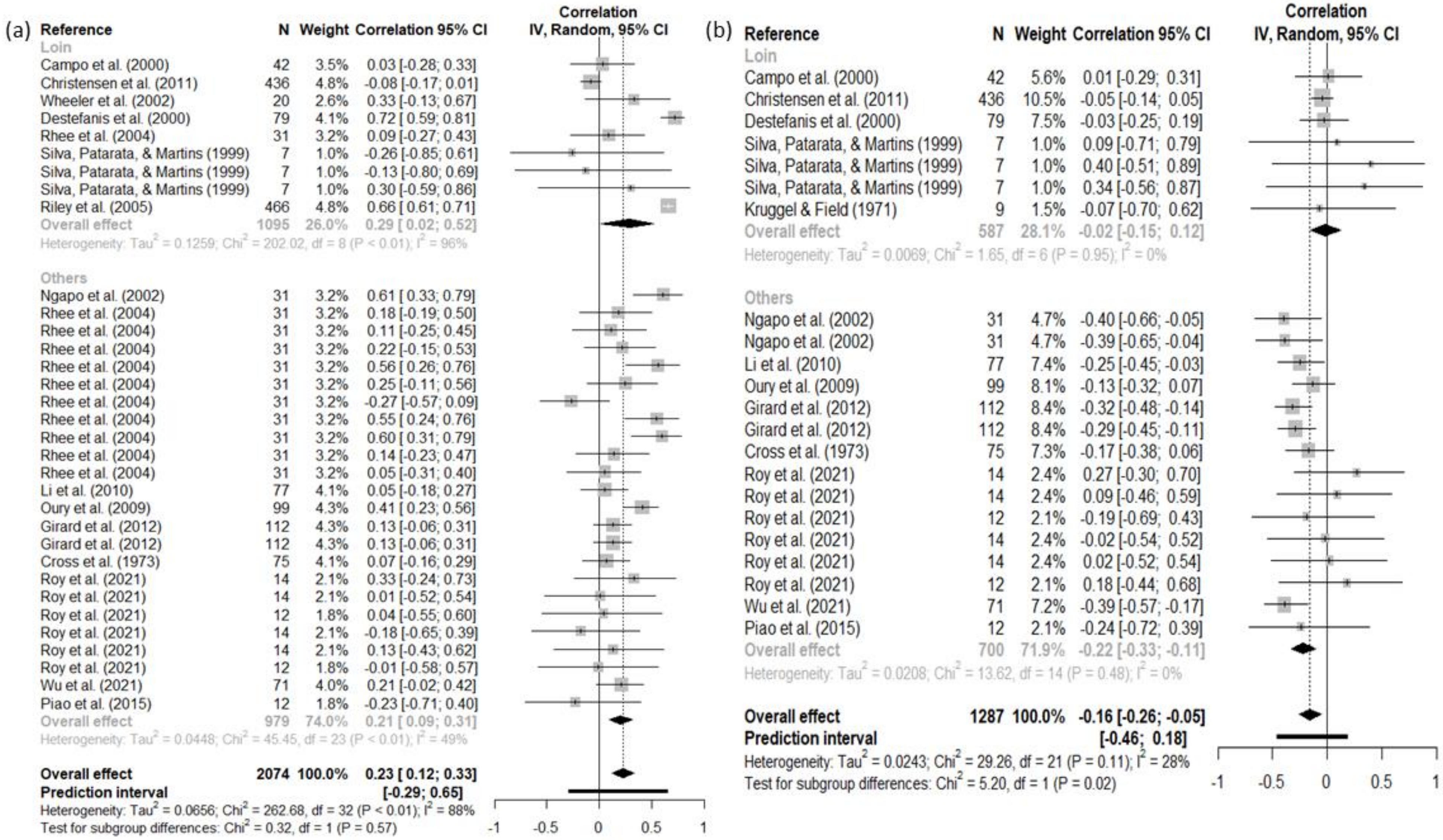
		Collagen content			Collagen solubility			Pyridinoline Cross-link		
	Age[†]	n	Slope[‡]	R^2 (%)	n	Slope[‡]	R^2 (%)	n	Slope[‡]	R^2 (%)
WBSF	Young	70	1.21±0.631	82.0	57	-0.32±0.215	83.7	8	5.5±72.6	53.2
	Old	28	2.48±1.11*	86.1	23	0.24±0.231	87.7	14	24.5±46.7	72.9
Sensory tenderness	Young	34	0.301±0.106**	46.8	17	-0.057±0.0695	66.5	12	3.9±11.5	58.0
	Old	21	-0.515±0.115***	77.1	17	-0.16±0.114	40.3	11	-11.03±8.94	6.8

[†] Young = animals with age <18 months, old = animals with age ≥18 months. *$p<0.05$, **$p<0.01$, ***$p<0.001$. [‡] Model: $y = \beta_0 + \beta_1 Col + \sum \beta_i R_i + \varepsilon$, where y is WBSF or sensory tenderness, Col is collagen content, solubility or pyridinoline cross-link content, and $\sum \beta_i R_i$ is the effect of references. R^2 is the percentage of variance explained by the model.

Table 4. Regression coefficient (slope, mean ± standard error), number of measurements (n) and R-squared of the regression model of beef tenderness attributes Warner-Bratzer shear force (WBSF, N) and sensory tenderness (1 = tough to 10 = tender) when both collagen content (mg/g fresh meat) and collagen solubility (%) are included together in the model

		Slope[†]		
Tenderness attributes	n	Collagen content	Collagen solubility	R^2 (%)
WBSF	91	1.75±0.628**	-0.54±0.145***	77.7
Sensory tenderness	41	-0.22±0.080*	0.082±0.0294*	61.0

[†] Model: $y = \beta_0 + \beta_1 TCol + \beta_2 ColS + \sum \beta_i R_i + \varepsilon$, where y is WBSF or sensory tenderness, TCol is collagen content, ColS is collagen solubility, and $\sum \beta_i R_i$ is the effect of references, *$p<0.05$, **$p<0.01$, ***$p<0.001$. R^2 is the percentage of variance explained by the model.



(a)

Reference	N	Weight	Correlation 95% CI
Loin			
Campo et al. (2000)	42	3.5%	0.03 [-0.28; 0.33]
Christensen et al. (2011)	436	4.8%	-0.08 [-0.17; 0.01]
Wheeler et al. (2002)	20	2.6%	0.33 [-0.13; 0.67]
Destefanis et al. (2000)	79	4.1%	0.72 [0.59; 0.81]
Rhee et al. (2004)	31	3.2%	0.09 [-0.27; 0.43]
Silva, Patarata, & Martins (1999)	7	1.0%	-0.26 [-0.85; 0.61]
Silva, Patarata, & Martins (1999)	7	1.0%	-0.13 [-0.80; 0.69]
Silva, Patarata, & Martins (1999)	7	1.0%	0.30 [-0.59; 0.86]
Riley et al. (2005)	466	4.8%	0.66 [0.61; 0.71]
Overall effect	1095	26.0%	0.29 [0.02; 0.52]
Heterogeneity: $Tau^2 = 0.1259$; $Chi^2 = 202.02$, df = 8 ($P < 0.01$); $I^2 = 96\%$			
Others			
Ngapo et al. (2002)	31	3.2%	0.61 [0.33; 0.79]
Rhee et al. (2004)	31	3.2%	0.18 [-0.19; 0.50]
Rhee et al. (2004)	31	3.2%	0.11 [-0.25; 0.45]
Rhee et al. (2004)	31	3.2%	0.22 [-0.15; 0.53]
Rhee et al. (2004)	31	3.2%	0.56 [0.26; 0.76]
Rhee et al. (2004)	31	3.2%	0.25 [-0.11; 0.56]
Rhee et al. (2004)	31	3.2%	-0.27 [-0.57; 0.09]
Rhee et al. (2004)	31	3.2%	0.55 [0.24; 0.76]
Rhee et al. (2004)	31	3.2%	0.60 [0.31; 0.79]
Rhee et al. (2004)	31	3.2%	0.14 [-0.23; 0.47]
Rhee et al. (2004)	31	3.2%	0.05 [-0.31; 0.40]
Li et al. (2010)	77	4.1%	0.05 [-0.18; 0.27]
Oury et al. (2009)	99	4.3%	0.41 [0.23; 0.56]
Girard et al. (2012)	112	4.3%	0.13 [-0.06; 0.31]
Girard et al. (2012)	112	4.3%	0.13 [-0.06; 0.31]
Cross et al. (1973)	75	4.1%	0.07 [-0.16; 0.29]
Roy et al. (2021)	14	2.1%	0.33 [-0.24; 0.73]
Roy et al. (2021)	14	2.1%	0.01 [-0.52; 0.54]
Roy et al. (2021)	12	1.8%	0.04 [-0.55; 0.60]
Roy et al. (2021)	14	2.1%	-0.18 [-0.65; 0.39]
Roy et al. (2021)	14	2.1%	0.13 [-0.43; 0.62]
Roy et al. (2021)	12	1.8%	-0.01 [-0.58; 0.57]
Wu et al. (2021)	71	4.0%	0.21 [-0.02; 0.42]
Piao et al. (2015)	12	1.8%	-0.23 [-0.71; 0.40]
Overall effect	979	74.0%	0.21 [0.09; 0.31]
Heterogeneity: $Tau^2 = 0.0448$; $Chi^2 = 45.45$, df = 23 ($P < 0.01$); $I^2 = 49\%$			
Overall effect	**2074**	**100.0%**	**0.23 [0.12; 0.33]**
Prediction interval			**[-0.29; 0.65]**

Heterogeneity: $Tau^2 = 0.0656$; $Chi^2 = 262.68$, df = 32 ($P < 0.01$); $I^2 = 88\%$

Test for subgroup differences: $Chi^2 = 0.32$, df = 1 ($P = 0.57$)

(b)

Reference	N	Weight	Correlation 95% CI
Loin			
Campo et al. (2000)	42	5.6%	0.01 [-0.29; 0.31]
Christensen et al. (2011)	436	10.5%	-0.05 [-0.14; 0.05]
Destefanis et al. (2000)	79	7.5%	-0.03 [-0.25; 0.19]
Silva, Patarata, & Martins (1999)	7	1.0%	0.09 [-0.71; 0.79]
Silva, Patarata, & Martins (1999)	7	1.0%	0.40 [-0.51; 0.89]
Silva, Patarata, & Martins (1999)	7	1.0%	0.34 [-0.56; 0.87]
Kruggel & Field (1971)	9	1.5%	-0.07 [-0.70; 0.62]
Overall effect	587	28.1%	-0.02 [-0.15; 0.12]
Heterogeneity: $Tau^2 = 0.0069$; $Chi^2 = 1.65$, df = 6 ($P = 0.95$); $I^2 = 0\%$			
Others			
Ngapo et al. (2002)	31	4.7%	-0.40 [-0.66; -0.05]
Ngapo et al. (2002)	31	4.7%	-0.39 [-0.65; -0.04]
Li et al. (2010)	77	7.4%	-0.25 [-0.45; -0.03]
Oury et al. (2009)	99	8.1%	-0.13 [-0.32; 0.07]
Girard et al. (2012)	112	8.4%	-0.32 [-0.48; -0.14]
Girard et al. (2012)	112	8.4%	-0.29 [-0.45; -0.11]
Cross et al. (1973)	75	7.3%	-0.17 [-0.38; 0.06]
Roy et al. (2021)	14	2.4%	0.27 [-0.30; 0.70]
Roy et al. (2021)	14	2.4%	0.09 [-0.46; 0.59]
Roy et al. (2021)	12	2.1%	-0.19 [-0.69; 0.43]
Roy et al. (2021)	14	2.4%	-0.02 [-0.54; 0.52]
Roy et al. (2021)	14	2.4%	0.02 [-0.52; 0.54]
Roy et al. (2021)	12	2.1%	0.18 [-0.44; 0.68]
Wu et al. (2021)	71	7.2%	-0.39 [-0.57; -0.17]
Piao et al. (2015)	12	2.1%	-0.24 [-0.72; 0.39]
Overall effect	700	71.9%	-0.22 [-0.33; -0.11]
Heterogeneity: $Tau^2 = 0.0208$; $Chi^2 = 13.62$, df = 14 ($P = 0.48$); $I^2 = 0\%$			
Overall effect	**1287**	**100.0%**	**-0.16 [-0.26; -0.05]**
Prediction interval			**[-0.46; 0.18]**

Heterogeneity: $Tau^2 = 0.0243$; $Chi^2 = 29.26$, df = 21 ($P = 0.11$); $I^2 = 28\%$

Test for subgroup differences: $Chi^2 = 5.20$, df = 1 ($P = 0.02$)

Figure 1. Forrest plots of subgroup analysis of (a) correlation between collagen content and WBSF, and (b) correlation between collagen solubility and WBSF. WBSF = Warner-Braztler shear force, loin = *Longissimus thoracis* or *lumborum*, others = *Adductor, Biceps brachii, Biceps femoris, Deep*

digital flexor, Extensor carpi radialis, Flexor digitorum, Gastrocnemius, Gluteobiceps, Gluteus medius, Infraspinatus, Long digital extensor, Medial digital extensor, Peroneus tertius, Psoas major, Rectus abdominis, Rectus femoris, Semimembranosus, Semitendinosus, Supraspinatus, Tensor fascia latae and Triceps brachii.

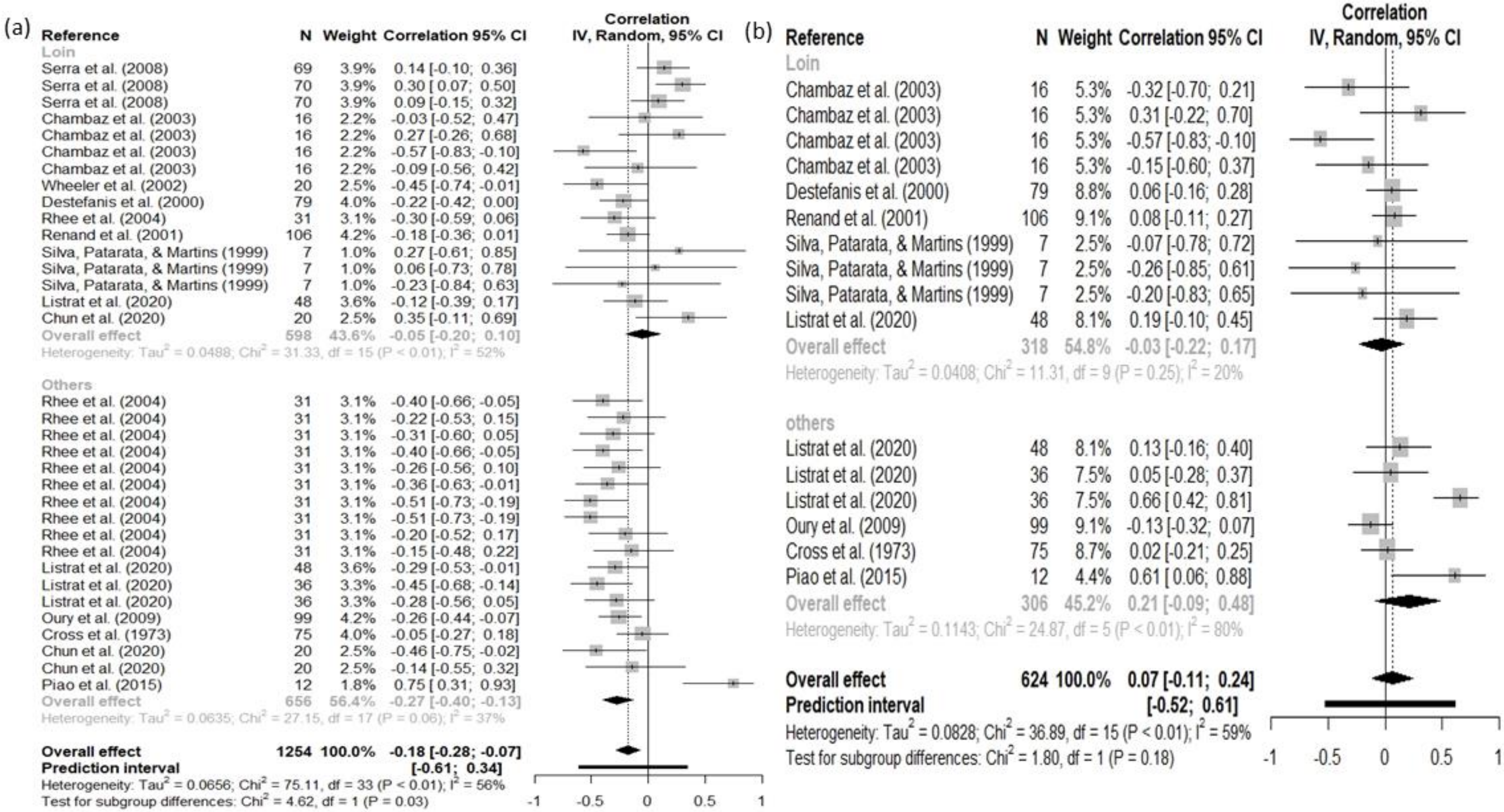


Figure 2. Forrest plots of subgroup analysis of (a) correlation between collagen content and sensory tenderness, and (b) correlation between collagen solubility and sensory tenderness. WBSF = Warner-Braztler shear force, loin = *Longissimus thoracis* or *lumborum*, others = *Adductor, Biceps brachii, Biceps femoris, Deep digital flexor, Extensor carpi radialis, Flexor digitorum, Gastrocnemius, Gluteobiceps, Gluteus medius, Infraspinatus, Long digital extensor, Medial digital extensor, Peroneus tertius, Psoas major, Rectus abdominis, Rectus femoris, Semimembranosus, Semitendinosus, Supraspinatus, Tensor fascia latae and Triceps brachii*.