DOI: https://doi.org/10.21323/2414-438X-2022-7-4-265-272

Available online at https://www.meatjournal.ru/jour
Original scientific article
Open Access

Received 15.11.2022
Accepted in revised 25.11.2022
Accepted for publication 01.12.2022

STUDY OF ADIPOSE TISSUE OF KEMEROVO PIGLETS: DETECTION OF BEIGE ADIPOCYTES

Viktoriya A. Pchelkina[1]*, Irina M. Chernukha[1], Igor A. Korotkiy[2], Nikolai A. Ilyin[1]
[1] V.M.Gorbatov Federal Research Center for Food Systems of RAS, Moscow, Russia
[2] Kemerovo State University, Kemerovo, Russia

Keywords: *pigs, histology, white adipocytes, beige adipocytes, localization, morphometry, Raman spectroscopy, fatty acid composition*

Abstract

Animals have two types of adipose tissue differing in structure and function: white (WAT) and brown (BAT). Beige adipose tissue (BeAT) is a result of WAT browning, when beige adipocytes appear between white adipocytes in response to exposure to cold, diet or physical activity. BeAT shares morphological and biochemical characteristics with BAT, is thermogenic and dissipates energy in the form of heat, unlike WAT, which is responsible for energy storage. Pigs do not have classic BAT, and modern breeds are sensitive to cold. There is literature information that BeAT has been found in cold-resistant pigs. The aim of the work was to study adipose tissue of piglets of the Kemerovo cold-resistant breed under growing conditions in the cold season and to identify the localization of BeAT. Histological studies have shown two types of adipocytes in subcutaneous fat samples (lateral, backfat and axillary): white, with one large fat droplet, and beige, multilocular. Larger cells were detected in backfat fat (69.36 ± 12.98 μm) compared with lateral (53.25 ± 9.27 μm) and axillary fat (45.94 ± 8.29 μm). Only WAT with a diameter of 35.69 ± 6.96 μm was present in the internal perirenal fat. Raman spectroscopy was used to evaluate the overall fatty acid profile of the tested samples. The main peaks were noted in all samples: 970 cm^{-1} (=C-H out-of-plane bend), 1266/1272 cm^{-1} (=C-H symmetrical rock) and 1655 cm^{-1} (C=C stretching) responsible for unsaturated bonds, and signals at 1297/1301 cm^{-1} (CH_2 twisting), 1430/1460 cm^{-1} (CH_2 symmetrical deformation (scissoring)) and 1735/1746 cm^{-1} (C=O stretching) corresponding to saturated bonds or ester groups. Internal perirenal fat contained the largest number of saturated fatty acid bonds, subcutaneous axillary fat — the smallest. The average intensity of the peaks was 0.4801010 and 0.639995, respectively. According to the results of gas chromatography, the largest amount of polyunsaturated fatty acids was noted in the subcutaneous fat samples: 20.199 in backfat fat, 21.749 in lateral and 20.436 in axillary fat compared to 18.636 in internal fat. Activation of beige cells in Kemerovo pigs under cold exposure, according to the authors, plays a crucial role in the heat balance, allowing them to tolerate cold without severe shivering. The study of the BeAT formation is of great practical importance for changing energy metabolism and increasing thermogenesis in newborn piglets by genome editing, as well as for improving the quality of pig's fat.

For citation: Pchelkina, V.A., Chernukha, I.M., Korotkiy, I.A., Ilyin, N.A. (2022). Study of adipose tissue of Kemerovo piglets: Detection of beige adipocytes. *Theory and Practice of Meat Processing*, 7(4), 265-272. https://doi.org/10.21323/2414-438X-2022-7-4-265-272

Funding:
This work was financially supported by the Russian Science Foundation (RSF) (project No. 21–76–20032).

Introduction

Adipose tissue is a metabolically active organ playing a key role in regulation of energy homeostasis of the body, including thermogenesis, takes part in the glucose metabolism, insulin secretion and regulation of immune reactions, secretes several bioactive peptides (adipokines) [1,2].

Based on the physiological functions, morphology and visual color, mammalian adipose tissue is divided into two types: white (WAT) and brown (BAT) [3]. WAT is located mainly under the skin and around internal organs. Cells contain a large unilocular lipid droplet and a small number of mitochondria. BAT is present in the embryonic period and during winter dormancy; its cells have many small multilocular lipid droplets and a large number of mitochondria. Beige adipose tissue (BeAT) is a result of WAT browning, when beige adipocytes appear between white adipocytes in response to exposure to several factors (for example, cold, diet or physical activity) [4]. Beige adipocytes have many common morphological and biochemical characteristics with brown adipocytes, including multilocular lipid droplets. Both brown and beige adipocytes are thermogenic and dissipate energy as heat, while white adipocytes are responsible for energy storage (Figure 1) [8].

Domestic pigs are an important resource in agriculture worldwide. Four main fat depots are differentiated in pigs: visceral, subcutaneous, intermuscular and intramuscular [6]; each of them has specific morphological and metabolic properties [7]. According to data from

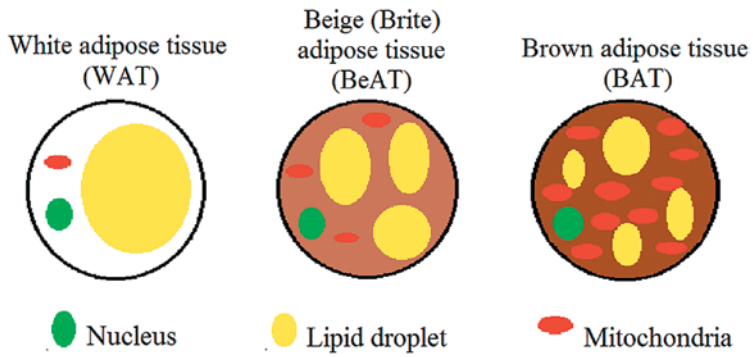

Figure 1. Comparison of three types of adipocytes [8]

literature sources, pigs do not have classic BAT despite the presence of brown adipocytes in the majority of mammals [8]. Thus, modern breeds are sensitive to cold, which is the main cause of mortality among piglets in pig husbandry [9]. However, there is information that beige adipocytes were found in axillary, inguinal subcutaneous, and perirenal WAT of cold-resistant Tibetan pigs and Min pigs [10].

The Kemerovo breed was selected in the Kemerovo region (Western Siberia) by crossing local pigs with sires of the Long-eared White, Large White, Large Black, Berkshire, Siberian North and Siberian Black-and-white breeds. The breed was approved in 1960 [11]. Nowadays, Kemerovo pigs are raised on the territory of Siberia and the Far East; however, the population is not large. It is significant that animals of this breed are distinguished by cold resistance and good adaptation to local climatic conditions, high viability and calm temper, intensive growth, sound constitution and early maturation. It is expedient to use these qualities in pig breeding to select new breeds and crosses in regions with sharp climate fluctuations and seasonal feeding base. Kemerovo pigs fall into the meat-and-lard (universal) direction of productivity and according to various literature data, a backfat size (at a level of 6^{th} –7^{th} thoracic vertebrae at a live weight of 100 kg) will be from 27–29 mm [12] to 35.8 ± 1.65 mm [13]. According to the data of Bekenev V. A. [14], backfat of Kemerovo pigs is characterized by an increased content of α-linolenic and docosahexaenoic fatty acids, a high PUFA: SFA ratio (0.12), which indicates its low fusibility, good palatability and high technological properties.

Several publications of both national and foreign authors are devoted to the study of adipose tissue of different pig breeds. The main attention in them is paid to the technological and consumer characteristics of raw materials obtained from adult (5–6 months of age) animals. Serra et al. [15] showed differences in thickness and fatty acid composition of backfat in Iberian and Landrace pigs with the same slaughter weight (105–118 kg). Iberian pigs were characterized by thicker backfat (48.1 versus 20.7 mm) that contained more saturated (SFA) and monounsaturated (MUFA) fatty acids and low concentrations of linoleic and linolenic fatty acids. Similar results but for Creole and Large White pigs are presented in [16]. In [17,18] characteristics of backfat for adult Basque and Large White pigs were compared: the Basque breed was characterized by earlier and higher development of adipose tissue (backfat depth was 26 versus 17 mm), as well as the high activity of enzymes responsible for lipid synthesis compared to the Large White animals. Nakajima et al. [19] showed that hypertrophy of adipocytes makes the highest contribution to the backfat depth and Kojima et al. [20] established differences in genome-wide expression profiles in adipose tissue in lard-type Meishan pigs compared to Landrace pigs. In [21] a relationship between the mRNA signaling pathway with subcutaneous adipogenesis and backfat thickness was shown by the example of Chinese Jiaxing pigs and White Large pigs. However, there is little information about the structure of the fat depot of difference loci and revelation of different types of adipocytes in them, especially in young individuals. Studies of functional and morphological properties of adipose tissue are especially important to extend geography of pig husbandry and prevent neonatal mortality. Therefore, it was interesting to study adipose tissue from pigs of domestic cold-resistant breeds (by the example of the Kemerovo breed) at the initial (juvenile) stage of the postembryonal development under conditions of raising in the cold season to reveal BeAT localization.

Objects and methods

Samples of adipose tissue of different localization from three-week-old Kemerovo piglets (n=3) kept under free-range conditions (private farm, Kemerovo) were studied. The samples included backfat (B), lateral (L), axillary fat (A), and internal perirenal fat (P) (Figure 2). The number of samples from one localization was no less than ten.

Animals were slaughtered according to the conventional techniques by cutting the jugular vein and the following bleeding. All manipulations were carried out according to the Directive 2010/63/EU of the European Parliament and of the Council [22], European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes (ETS No. 123) [23], Recommendations for euthanasia of experimental animals: Part 1. Laboratory Animals (1996) 30, Recommendations

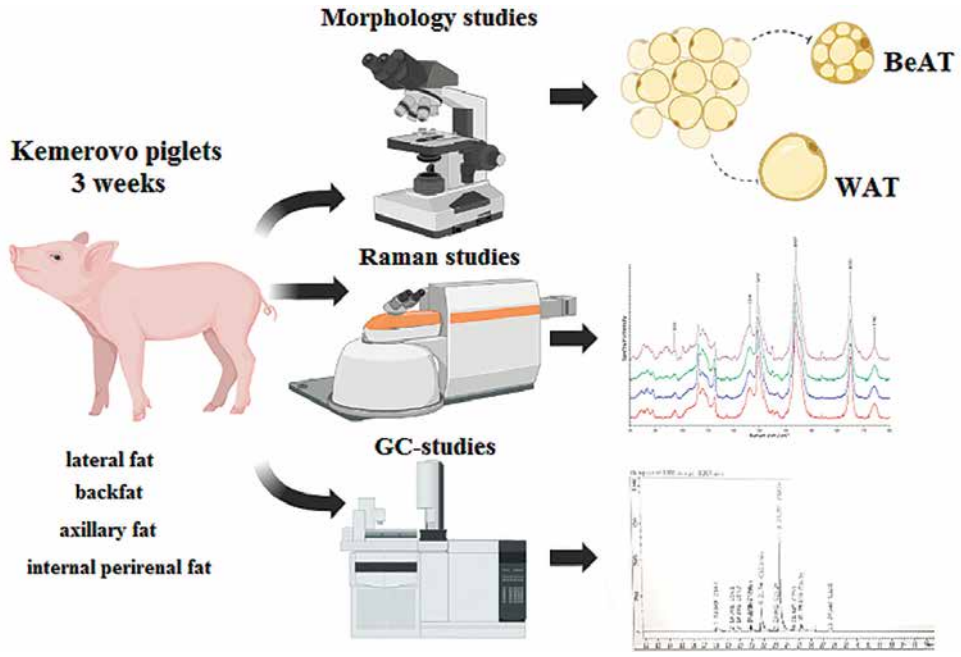

Figure 2. Graphic illustration of the performed study

for euthanasia of experimental animals: Part 2. Laboratory Animals (1997) 31, 1–32), as well as requirements of GOST 33215–2014[1]

Histological studies

To study morphology of adipose tissue, samples were fixed in the 10% neutral buffered formalin solution (BioVitrum, Russia) for 72 hour at room temperature, washed with cold running water for four hours and embedded in gelatin (AppliChem GMBH, Germany) in an ascending concentration (12.5%, 25%) at a temperature of 37 °C for 8 hours each using a thermostat TS-1/20 SPU (Smolensk SKTB-SPU, Russia). Sections with a thickness of 12 μm were made on the cryostat «MIKROM–HM525» (Thermo Scientific, USA). The obtained sections were mounted on Menzel-Glaser slides (Thermo Scientific, USA) and stained with Ehrlich's hematoxylin and 1% aqueous-alcoholic solution of eosin (BioVitrum, Russia) by the conventional method [24]. The histological preparations were studied using an Axio Imager A1 light microscope (Carl Zeiss, Germany).

To count a diameter of adipocytes, the modified method [25] was applied: sections with a thickness of 12 μm were made from the samples fixed in formalin, mounted on slides, put in a droplet of the physiological solution under a cover glass and immediately analyzed using the image analysis system AxioVision 4.7.1.0 (Carl Zeiss, Germany). No less than three sections were made from each piece. A diameter of adipocytes was measured for 100 cells in each section in the interactive mode with an accuracy of ± 0.1 μm.

Raman spectroscopy

Raman spectroscopy was used to assess the overall fatty acid profile of tested samples [26]. Collection of spectra was carried out on the confocal Raman dispersive spectrometer Renishaw (model inVia Reflex, Renishaw plc, Wotton-under-Edge, UK). A laser with a wavelength of 785 nm, power of 100 mW and exposure time of 10s was used. Power of laser radiation and time of integration were thoroughly optimized to prevent photodegradation of fat samples. Calibration of the spectrometer was performed by registration of the silicon spectrum at 520 cm^{-1}. Raman spectra were obtained directly from samples of adipose tissue with a size of 10 × 10 × 5 mm. For laser focusing, an objective with a magnification of L50× was used. Measurements were recorded in a detection range of 700–1800 cm^{-1}. No less than six spectra were recorded for each sample.

All collected spectra were subjected to preliminary treatment (cosmic ray removal, baseline correction, smoothing using the algorithm of Savitzky-Golay, normalization) and analysis using the software Renishaw WiRE 5.2 (Renishaw plc, Wotton-under-Edge, UK).

Gas chromatography

For chromatographic analysis, a sample was rendered on an IKA Hot Plate C–MAG HP 7 (IKA®-Werke GmbH & Co. KG, Germany). To obtain fatty acid methyl esters, rendered fat was taken in a quantity of 200 μl and transferred into a 15 ml centrifuge tube, 2 ml of 2M potassium hydroxide solution in methanol was added with the following addition of 4 ml of hexane; centrifugation was carried out at 3,000–5,000 rpm for 1–3 min. After centrifugation, 200 μl were taken from the upper hexane layer and transferred into a chromatographic vial; 800 μl of pure hexane was added to dilute concentrations of fatty acids. The obtained sample was analyzed on a gas chromatograph Agilent

[1] GOST 33215–2014 "Guidelines for accommodation and care of animals. Environment, housing and management" Moscow: Standartinform, 2019. Retrieved from https://docs.cntd.ru/document/1200127789 Accessed September 16, 2022

7890 (Agilent Technologies, Inc., USA) with a flame ionization detector using a capillary column Agilent HP 5 30 m × 0.32 mm × 0.25 μm (carrier gas: nitrogen) (Agilent Technologies, Inc., USA).

Statistical processing of results

Statistical data analysis was carried out using a package STATISTICA, version 10.0 (StatSoft, Inc., USA). The results of morphometry were presented as root mean square (S), root-mean-square (standard) deviation (±SD), minimum and maximum values [MIN MAX] of the interquartile range (P 25/75). For analysis of Raman spectra, the geometric mean (*GeoMean*) was used. Differences were considered significant and a relationship between parameters was acknowledged at a probability level of not higher than 0.05.

Results and discussion

In histological examination of samples taken from adipose tissue of different localization, it was found that two cell types are present in the samples of subcutaneous B, L and A fat: 1 — round, with one large fat droplet and flattened nucleus shifted to periphery, that correspond to white adipocytes by structure and 2 — having a polygonal shape and several fat droplets with different sizes (from 2 to 15 μm in diameter) corresponding to beige adipocytes by their structure [5] (Figures 3A, 3B). Connective tissue interlayers with the large number of fibrous elements were located between adipocytes. In the internal perirenal fat, cells were characterized by more round shape; the presence of beige adipocytes was not revealed. However, according to data [9] their formation in this locus was noticed in the cold-resistant Tibetan and Min pig breeds.

It is known that subcutaneous WAT is especially prone to the BeAT development in the periods of adaptive thermogenesis [8]. Lin et al. [9] found beige adipocytes in white adipose tissue of pigs exposed to cold. In our research, samples of adipose tissue were taken from piglets kept under free-range conditions during winter in the natural temperature conditions without additional heating, which led to the development of BeAT in WAT. Probably, this is linked with the fact that the mechanism of UCP3-dependent thermogenesis in beige adipocytes has been evolutionally developed in cold-resistant pigs [8,10].

As known, on early life stages, porcine adipose tissue grows mainly due to hyperplasia (an increase in the number) of adipocytes [6,7]. After a significant increase in the cell number, adipocytes begin to increase in size (hypertrophy) due to accumulation of triglycerides [27]. In our samples, an average diameter of adipocytes was as follows: 69.36 ± 12.98 μm in backfat, 53.25 ± 9.27 in lateral, 45.94 ± 8.29 in axillary fat, 35.69 ± 6.96 μm in internal perirenal fat. Our results agree with the results of other authors regarding sizes of adipocytes in piglets. According to [19], an average diameter of adipocytes in backfat in three-week-old piglets of the Landrace bacon breed was 53.4 ± 3.6 μm and of the lard-type Meishan breed 58.7 ± 7.4 μm.

It was established that a size of adipocytes of subcutaneous fat changes depending on the location on the animal body — larger cells were revealed in backfat fat samples compared to lateral and axillary fat (Figure 3C). It is linked with the most intensive development of fat in this locus. A wide range of adipose cell sizes and the presence of "small" adipocytes also noticed in other pig breeds [19] allow suggesting that both cell hyperplasia and hypertrophy are still active. Adipose tissue in all studied loci is in the intensive growth period and there is the potential for the following filling of cells with lipids and increase in the thickness of subcutaneous fat. A proportion of "small" adipocytes was 18–20%.

Adipose tissue in pigs is the main place of lipid synthesis, i. e. lipogenesis, during which adipocytes synthesize and accumulate triglycerides and provide no less than 80% of deposited fatty acids [6,28]. Today, quick and non-destructive methods for assessing overall fatty acid profile that allow performing analysis directly in production are in demand. We used Raman spectroscopy, which belongs to such screening methods [29].

Raman spectra of pork fat are presented by signals conditioned by vibrations of hydrocarbon chains in saturated and unsaturated structures [30]. The main peaks responsible for unsaturated bonds were 970 cm^{-1} (=C-H out-of-plane bend), 1266/1272 cm^{-1} (=C-H symmetric rock) and 1655 cm^{-1} (C=C stretching); while signals at 1297/1301 cm^{-1} (CH_2 twisting), 1430/1460 cm^{-1} (CH_2 symmetric deformation (scissoring)) and 1735/1746 cm^{-1} (C=O stretching) correspond to saturated bonds or ester groups [31,32]. The obtained spectra of adipose tissue samples are presented in Figure 1D. Differences between samples in the intensity of the main signals were revealed. To determine the relative content of the unsaturated bonds, an intensity ratio of the main signals corresponding to unsaturated bonds, and signals of saturated bonds was calculated in each sample. For calculation, nine intensity ratios were used: I_{970}/I_{1297}, I_{970}/I_{1430}, I_{970}/I_{1735}, I_{1266}/I_{1297}, I_{1266}/I_{1430}, I_{1266}/I_{1735}, I_{1655}/I_{1297}, I_{1655}/I_{1430}, I_{1655}/I_{1735} (Table 1).

Table 1. Relative content of unsaturated bonds in the tested adipose tissue samples

Intensity (I) ratio of Raman signals	Lateral fat	Backfat	Axillary fat	Internal perirenal fat
I_{970}/I_{1297}	0.130821	0.112026	0.159802	0.090597
I_{970}/I_{1430}	0.077807	0.069489	0.092581	0.062879
I_{970}/I_{1735}	0.546442	0. 75685	0.722015	0.471012
I_{1266}/I_{1297}	0.55442	0.501723	0.580229	0.40954
I_{1266}/I_{1430}	0.3333	0.311286	0.338914	0.284375
I_{1266}/I_{1735}	2.329259	2.1305	2.635744	2.129262
I_{1655}/I_{1297}	1.098959	1.03814	1.068285	0.82681
I_{1655}/I_{1430}	0.659668	0.644123	0.626257	0.573635
I_{1655}/I_{1735}	4.613256	4.408478	4.863001	4.29427
GeoMean	0.584857	0.535684	0.639995	0.480101

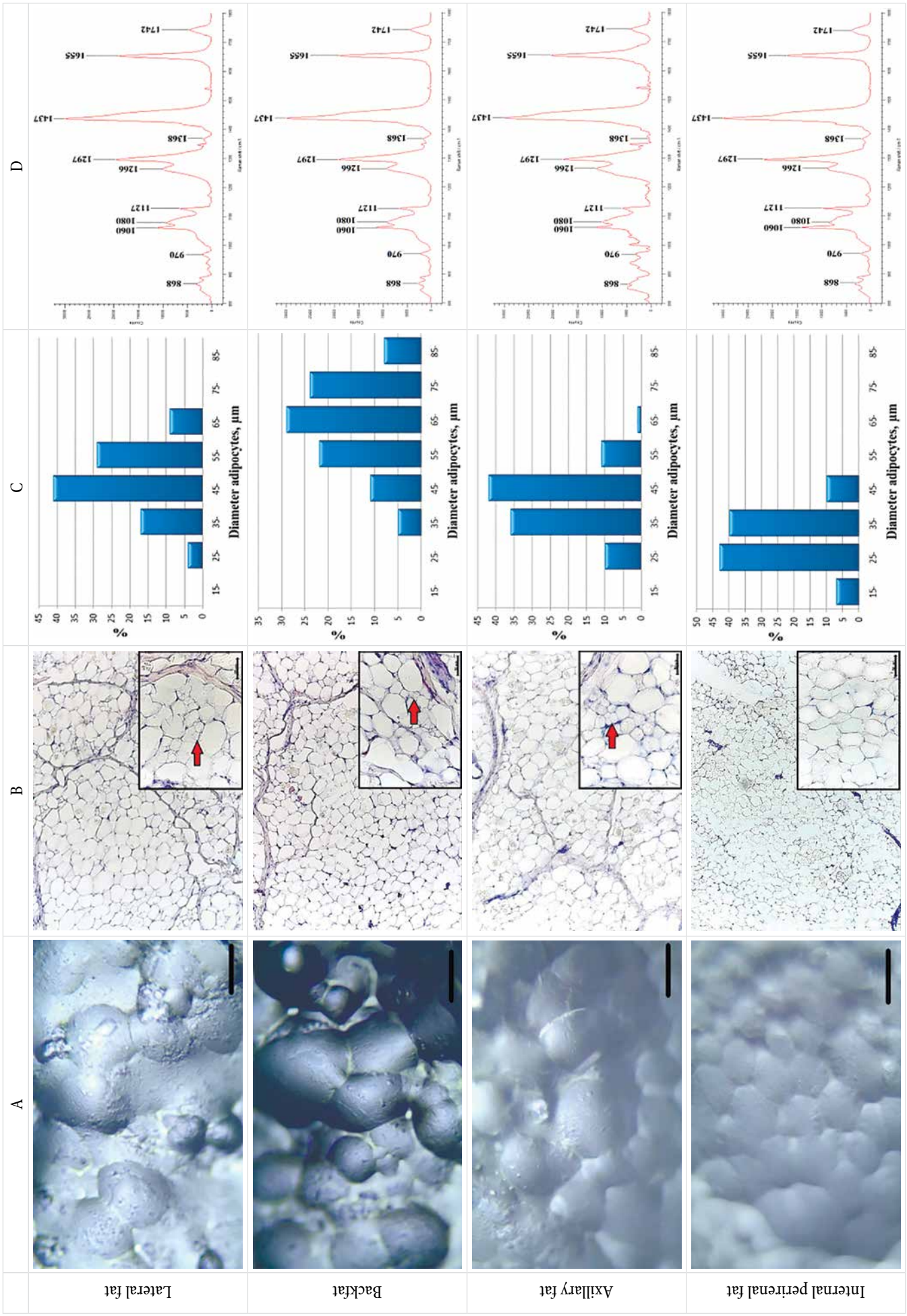

Figure 3. Effect of localization on characteristics of adipose tissue of Kemerovo piglets: a. Morphology of samples without staining (Scale bar — 50 μm); B — Morphology of samples after staining with hematoxylin and eosin (Scale bar — 50 μm). The arrow indicates beige adipocytes; C — Distribution of adipocytes by diameter; D — Raman spectra of the samples

The average intensity of peaks responsible for unsaturated bonds was lower in internal perirenal fat than in subcutaneous fat and was equal to 0.480101. This indicates that it contains lower amounts of unsaturated fatty acids and higher amounts of saturated fatty acids. Among samples of subcutaneous adipose tissue, backfat contained more saturated bonds compared to lateral and axillary fat. The highest average intensity of peaks responsible for unsaturated bonds (0.639995) was in axillary fat. As the difference between certain fatty acids resides in the length of carbon chains as well as the number and position of double bonds, they have similar Raman spectra [33,34]. Therefore, a problem of overlapping peaks from different fatty acids arises in analysis of adipose tissue samples [35]. In this connection, detection of certain fatty acids presents difficulties.

By the results of gas chromatographic analysis of the fatty acid composition, ΣSFA in all samples did not show significant differences and was 35.161 in internal perirenal fat and 35.144 in subcutaneous fat. The main detected saturated fatty acids were palmitic, stearic, margaric and myristic acids. The sum of UFA, the most important of which were oleic, palmitoleic, linoleic and linolenic acids, was 60.923 in the samples of internal perirenal fat and 61.095 in backfat. With that, the highest amounts of polyunsaturated fatty acids were found in the samples of subcutaneous fat: 20.199 in backfat, 21.749 in lateral and 20.436 in axillary fat compared to 18.636 in internal perirenal fat.

It is known that the fatty acid composition depends on fat localization in the animal body [7]. The results on the fatty acid composition in the fatty tissue samples of different localization that we have obtained are in agreement with the results of other authors. It is shown in [36] that the unsaturated fatty acid content in pigs changes according to the gradient: the highest content is in the external layer of subcutaneous adipose tissue, where less mature adipocytes are located followed by the internal layer, intermuscular and internal fat, where adipocytes are more mature. According to the opinion of Jiang et al. [37], this fact is linked with a higher Δ-9-desaturase activity index in external fat depots compared to internal and replacement of stearic fatty acid with oleic in them. Lee et al. [38] obtained similar results for three steer breeds and concluded that mature fat is more saturated than immature; therefore, a degree of unsaturation can be an index of its maturity.

Conclusion

The Kemerovo breed of pigs was selected in Siberia and, consequently, is adapted to local cold climate conditions. Histological analysis of adipose tissue of different localization taken from three-week-old piglets exposed to cold showed the presence of two types of adipocytes in subcutaneous fat (backfat, lateral and axillary): white having one large fat droplet and beige having several fat droplets with various sizes (multilocular). Larger cells were revealed in backfat samples compared to lateral and axillary fat. Only white adipocytes were found in the internal perirenal fat. In comparison of the fatty acid profile in the samples by the results of Raman spectroscopy, a larger quantity of saturated bonds was noticed in internal fat, while in subcutaneous fat, especially axillary, a larger quantity of unsaturated bonds was found. This finding was confirmed by the results of gas chromatography. Polyunsaturated fatty acids, linoleic and linolenic, dominated in the samples of subcutaneous fat.

Activation of beige cells in Kemerovo pigs on exposure to cold can play an important role in the heat balance allowing them to tolerate cold without severe shivering. Studying the development of beige adipocytes as well as mechanisms and factors inducing it can be of great importance for increasing thermogenesis in newborn piglets by genome editing. Beige adipogenesis in cold-resistant pigs opens a possibility of changing energy metabolism and improving quality of porcine adipose tissue.

REFERENCES

1. Ali, A. T., Hochfeld, W. E., Myburgh, R., Pepper, M. S. (2013). Adipocyte and adipogenesis. *European Journal of Cell Biology*, 92(6–7), 229–236. https://doi.org/10.1016/j.ejcb.2013.06.001
2. Cheng, L., Wang, J., Dai, H., Duan, Y., An, Y., Shi, L. et al. (2021). Brown and beige adipose tissue: a novel therapeutic strategy for obesity and type 2 diabetes mellitus. *Adipocyte*, 10(1), 48–65. https://doi.org/10.1080/21623945.2020.1870060
3. Chu, D.-T., Gawronska-Kozak, B. (2017). Brown and brite adipocytes: Same function, but different origin and response. *Biochimie*, 138, 102–105. https://doi.org/10.1016/j.biochi.2017.04.017
4. Pilkington, A.-C., Paz, H.A., Wankhade, U.D. (2021). Beige adipose tissue identification and marker specificity — Overview. *Frontiers in Endocrinology*, 12, Article 599134. https://doi.org/10.3389/fendo.2021.599134
5. Cohen, P., Kajimura, S. (2021). The cellular and functional complexity of thermogenic fat. *Nature Reviews Molecular Cell Biology*, 22(6), 393–409. https://doi.org/10.1038/s41580–021–00350–0
6. Poklukar, K., Čandek-Potokar, M., Lukač, N.B., Tomažin, U., Škrlep, M. (2020). Lipid deposition and metabolism in local and modern pig breeds: A review. *Animals,* 10(3), Article 424. https://doi.org/10.3390/ani10030424
7. Monziols, M., Bonneau, M., Davenel, A., Kouba, M. (2007). Comparison of the lipid content and fatty acid composition of intermuscular and subcutaneous adipose tissues in pig carcasses. *Meat Science*, 76(1), 54–60. https://doi.org/10.1016/j.meatsci.2006.10.013
8. Chernukha, I.M., Fedulova, L.V., Kotenkova, E.A. (2022). White, beige and brown adipose tissue: structure, function, specific features and possibility formation and divergence in pigs. *Foods and Raw Materials*, 10(1), 10–18. https://doi.org/10.21603/2308–4057–2022–1–10–18
9. Lin, J., Cao, C, Tao, C., Ye, R., Dong, M., Zheng, Q. et al. (2017). Cold adaptation in pigs depends on UCP3 in beige adipocytes. *Journal of Molecular Cell Biology*, 9(5), 364–375. https://doi.org/10.1093/jmcb/mjx018
10. Zhao, J., Tao, C., Chen, C., Wang, Y., Liu, T. (2021). Formation of thermogenic adipocytes: What we have learned from pigs. *Fundamental Research*, 1(4), 495–502. https://doi.org/10.1016/j.fmre.2021.05.004
11. Kharzinova, V.R., Zhuchaev, K.V., Kostyunina, O.V., Kochneva, M.L., Chydym, S.M., Zinovieva, N.A. (2017). Molecular-genetic identification and certification of Kemerovo breed of pigs based on STR- analysis. *Achievements of Science and Technology of the Agro-Industrial Complex,* 31(6), 62–64. (In Russian)
12. Comparative table for pig breeds. Meatinfo Retrieved from: https://meatinfo.ru/info/show?id=58. Accessed September 20, 2022 (In Russian)
13. Bekenev, V.A., Arishin, A.A., Mager, S.N., Bolshakova, I.V., Tretyakova, N.L., Kashtanova, E.V. at al. (2021). Lipid profile of pig tissues contrasting in meat production. *The Natural Products Journal*, 11(1), 108–118. https://doi.org/10.2174/2210315509666191203124902
14. Bekenev, V.A., Deeva, V.S., Arishin, A.A., Chernukha, I.M., Botan, I.V., Tretyakova, N.L. (2016). Usage of pig breeding bioresources in quality of pork. *Vestnik NSAU (Novosibirsk State Agrarian University)*, 3(40), 176–184 (In Russian)
15. Serra, X., Gil, F., Pérez-Enciso, M., Oliver, M.A., Vázquez, J.M., Gispert, M. et al. (1998). A comparison of carcass, meat quality and histochemical characteristics of Iberian (Guadyerbas line) and Landrace pigs. *Livestock Production Science*, 56(3), 215–223. https://doi.org/10.1016/S0301–6226(98)00151–1
16. Renaudeau, D., Mourot, J. (2007). A comparison of carcass and meat quality characteristics of Creole and Large White pigs slaughtered at 90kg BW. *Meat Science*, 76(1), 165–171. https://doi.org/10.1016/j.meatsci.2006.10.024
17. Alfonso, L., Mourot, J., Insausti, K., Mendizabal, J. A., Arana, A. (2005). Comparative description of growth, fat deposition, carcass and meat quality characteristics of Basque and Large White pigs. *Animal Research*, 54(1) 33–42. https://doi.org/10.1051/animres:2005001
18. Lebret, B., Dourmad, J.Y., Mourot, J., Pollet, P.Y., Gondret, F. (2014). Production performance, carcass composition, and adipose tissue traits of heavy pigs: Influence of breed and production system. *Journal of Animal Science,* 92(8), 3543–3556. https://doi.org/10.2527/jas.2013–7398
19. Nakajima, I., Oe, M., Ojima, K., Muroya, S., Shibata, M., Chikuni, K. (2011). Cellularity of developing subcutaneous adipose tissue in Landrace and Meishan pigs: Adipocyte size differences between two breeds. *Animal Science Journal*, 82(1), 144–149. https://doi.org/10.1111/j.1740–0929.2010.00810.x
20. Kojima, M., Nakajima, I., Arakawa, A., Mikawa, S., Matsumoto, T., Uenishi, H. et al. (2018). Differences in gene expression profiles for subcutaneous adipose, liver, and skeletal muscle tissues between Meishan and Landrace pigs with different backfat thicknesses. *PloS ONE*, 13(9), Article e0204135. https://doi.org/10.1371/journal.pone.0204135
21. Zhang, D., Wu, W., Huang, X., Xu, K., Zheng, C., Zhang, J. (2021). Comparative analysis of gene expression profiles in differentiated subcutaneous adipocytes between Jiaxing Black and Large White pigs. *BMC Genomics*, 22(1), Article 61. https://doi.org/10.1186/s12864–020–07361–9
22. Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes. Official Journal of the European Union, L 276/33 Retrieved from https://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ: L:2010:276:0033:0079: en: PDF Accessed September 16, 2022
23. European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes. European Treaty Series — No. 123. Retrieved from https://rm.coe.int/168007a67b Accessed September 16, 2022
24. Romeis B. (1989). Microscopic technique. München: Urban und Schwarzenberg, 1989. (In German)
25. Velotto, S., Claudia, V., Crasto, A. (2012). Muscle fibre types, fat deposition and fatty acid profile of Casertana versus Large White pig. *Animal Science Papers and Reports*, 30(1), 35–44.
26. Lee, J.-Y., Park, J.-H., Mun, H., Shim, W.-B., Lim, S.-H., Kim, M.-G. (2018). Quantitative analysis of lard in animal fat mixture using visible Raman spectroscopy. *Food Chemistry*, 254, 109–114. https://doi.org/10.1016/j.foodchem.2018.01.185
27. Louveau, I., Perruchot, M.-H., Bonnet, M., Gondret, F. (2016). Invited review: Pre- and postnatal adipose tissue development in farm animals: From stem cells to adipocyte physiology. *Animal,* 10(11), 1839–1847. https://doi.org/10.1017/S1751731116000872
28. Kloareg, M., Noblet, J., van Milgen, J. (2007). Deposition of dietary fatty acids, de novo synthesis and anatomical partitioning of fatty acids in finishing pigs. *British Journal of Nutrition,* 97(1), 35–44. https://doi.org/10.1017/S0007114507205793
29. Pchelkina, V.A., Chernukha, I.M., Fedulova, L.V., Ilyin, N.A. (2022). Raman spectroscopic techniques for meat analysis: A review. *Theory and Practice of Meat Processing*, 7(2), 97–111. https://doi.org/10.21323/2414–438X-2022–7–2–97–111
30. Beattie, J.R., Bell, S.E.J, Borgaard, C., Fearon, A., Moss, B.W. (2006). Prediction of adipose tissue composition using Raman spectroscopy: Average properties and individual fatty acids. *Lipids*, 41(3), 287–294. https://doi.org/10.1007/s11745–006–5099–1
31. Berhe, D.T., Eskildsen, C.E., Lametsch, R., Hviid, M.S., van den Berg, F., Engelsen, S.B. (2016). Prediction of total fatty acid parameters and individual fatty acids in pork backfat using Raman spectroscopy and chemometrics: Understanding the cage of covariance between highly correlated fat parameters. *Meat Science*, 111, 18–26. https://doi.org/10.1016/j.meatsci.2015.08.009
32. Saleem, M., Amin, A., Irfan, M. (2021). Raman spectroscopy based characterization of cow, goat and buffalo fats. *Journal of Food Science and Technology*, 58(1), 234–243. https://doi.org/10.1007/s13197–020–04535-x
33. Czamara, K., Majzner, K., Pacia, M., Kochan, K., Kaczor, A., Baranska, M. (2014). Raman spectroscopy of lipids: A review. *Journal of Raman Spectroscopy*, 46, 4–20. https://doi.org/10.1002/jrs.4607
34. De Gelder, J., De Gussem, K., Vandenabeele, P., Moens, L. (2007). Reference database of Raman spectra of biological molecules. *Journal of Raman Spectroscopy*, 38, 1133–1147. https://doi.org/10.1002/jrs.1734
35. Afseth, N.K., Dankel, K., Andersen, P.V., Difford, G.F., Horn, S.S., Sonesson, A. et al. (2022). Raman and near Infrared Spectroscopy for quantification of fatty acids in muscle tissue –A salmon case study. *Foods*, 11(7), Article 962. https://doi.org/10.3390/foods11070962
36. de Tonnac, A., Guillevic, M., Mourot, J. (2018). Fatty acid composition of several muscles and adipose tissues of pigs fed n-3 PUFA rich diets. *Meat Science*, 140, 1–8. https://doi.org/10.1016/j.meatsci.2017.11.023
37. Jiang, Q., Li, C., Yu, Y., Xing, Y., Xiao, D., Zhang, B. (2018). Comparison of fatty acid profile of three adipose tissues in Ningxiang pigs. *Animal Nutrition*, 4(3), 256–259. https://doi.org/10.1016/j.aninu.2018.05.006
38. Lee, J.-H., Yamamoto, I., Jeong, J.-S., Nade, T., Arai, T., Kimura, N. (2011). Relationship between adipose maturity and fatty acid composition in various adipose tissues of Japanese Black, Holstein and Crossbred (F1) steers. *Animal Science Journal*, 82, 689–697. https://doi.org/10.1111/j.1740–0929.2011.00893.x

AUTHOR INFORMATION

Viktoriya A. Pchelkina, Candidate of Technical Sciences, Leading Researcher, Experimental Clinic-Laboratory of Biologically Active Substances of Animal Origin, V. M. Gorbatov Federal Research Center for Food Systems. 26, Talalikhina str., 109316, Moscow, Russia. Tel.: +7–495–676–95–11 (242), E-mail: v.pchelkina@fncps.ru
ORCID: http://orcid.org/0000–0001–8923–8661
* corresponding author

Irina M. Chernukha, Doctor of Technical Sciences, Academician of RAS, Principal Researcher, Experimental Clinic-Laboratory of Biologically Active Substances of Animal Origin, V. M. Gorbatov Federal Research Center for Food Systems. 26, Talalikhina str., 109316, Moscow, Russia. Tel.: +7–495–676–95–11 (511), E-mail: imcher@inbox.ru
ORCID: http://orcid.org/0000–0003–4298–0927

Igor A. Korotkiy, Doctor of Technical Sciences, Professor, Head of the Department of Heat and Refrigeration Engineering, Kemerovo State University. 6, Krasnaya Str., 650056, Kemerovo, Russia. Tel.: +7–3842–73–43–44, E-mail: krot69@mail.ru
ORCID: http://orcid.org/0000–0002–7623–0940

Nikolai A. Ilyin, Senior Laboratory Assistant, Experimental Clinic-Laboratory of Biologically Active Substances of Animal Origin, V. M. Gorbatov Federal Research Center for Food Systems. 26, Talalikhina str., 109316, Moscow, Russia. Tel.: +7–495–676–95–11 (128),
E-mail: n.ilin@fncps.ru
ORCID: https://orcid.org/0000–0002–7980–3193

All authors bear responsibility for the work and presented data.

All authors made an equal contribution to the work.

The authors were equally involved in writing the manuscript and bear the equal responsibility for plagiarism.

The authors declare no conflict of interest.