FATTY ACID COMPOSITION OF SUBCUTANEOUS AND VISCERAL FAT DEPOTS IN NEW ZEALAND WHITE RABBITS

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Summary


The aim of this study was to identify the differences in the fatty acid composition of subcutaneous and visceral fat depots in healthy New Zealand White rabbits. Twelve clinically healthy rabbits with an average weight of 3.00±0.03 kg were used. The fatty acid composition of interscapular, inguinal, pericardial, perirenal and omental fat depots was determined by gas chromatography. The palmitic (C16:0) and linoleic (C18:2) acids, followed by oleic acid (C18:1) prevailed in all fat depots. The highest percentage of palmitic acid (C16:0) was detected in subcutaneous depots: inguinal (41.05±1.80%) and interscapular (38.30±0.73%), whereas the highest percentage of linoleic acid (C18:2) was found in the visceral depots: perirenal (44.26±0.96%) and pericardial (42.77±1.19%). Among the saturated fatty acids, myristic (C14:0) and stearic acid (C18:0) were established in higher content in subcutaneous depots than in visceral ones. Palmitoleic acid (C16:1) content in the pericardial fat depot was 10.63±2.60%, while in the interscapular, perirenal, omental and inguinal FD it was almost twice lower (P<0.001). In the omental depot, α-linolenic acid (C18:3) content was significantly higher only vs the interscapular depot (P<0.05). The high content of saturated fatty acids in the subcutaneous depots determined their higher atherogenic and saturation index, unlike visceral ones, where a significantly higher content of unsaturated fatty acids was reported. Differences in fatty acid composition of subcutaneous and visceral fat depots proved the specific metabolism in each of them. On the other hand, this led to differences in the nutritional value of various parts of rabbit carcass.

Key words: fatty acid composition, rabbits, subcutaneous adipose tissue, visceral adipose tissue
INTRODUCTION

Adipose tissue in rabbits is formed in subcutaneous and visceral depots (Cinti, 2005). The interscapular fat depot and inguinal fat depot are located subcutaneously, in the topographical regions with the same names (Blasco & Ouhayoun, 1993). Visceral depots are situated in thoracic, abdominal and pelvic cavities surrounding the relevant internal organs. These include pericardial, mesenteric, omental, perirenal, perivesical and perigonadal fat depots (Cinti, 2007; Iacobellis & Willens, 2009).

As in other monogastric animals, the dietary fatty acids (FAs) in rabbits are directly incorporated into the adipose tissue, almost unchanged. Long and medium chain FAs are catabolised as energy sources in adipocytes, while long-chained FAs are deposited as triglycerides (Ranganathan et al., 1995; Marks et al., 1996; Gondret et al., 1998). According to Peiretti et al. (2007), the FA composition of adipose tissues is in a direct relation to the influence of FA profile in the diet. FA profile depends on feeding mode (Oliver et al., 1997; Bernardini et al., 1999), age (Oriani et al., 2005), genotype, breeding and physical activity of the animals (Cobos et al., 1995; Nürnberg, et al., 1998; Banskalieva et al., 2000; Hu & Willett, 2002; De Smet et al., 2004). One of the main goals of nutritional researchers is improving the n-3 polyunsaturated fatty acids (PUFA) content, decreasing the n-6/n-3 ratio and reducing the saturation, atherogenic and thrombogenic indices of meat and fat. From this derive the benefits of the nutritional value of rabbit meat for consumers. The FA profiles of meat and fat can be effectively modified, when the rabbits are fed different dietary supplements. Enrichment of rations with oils derived from soybean, sunflower, rapeseed (Cobos et al., 1993), linseed (Kouba et al., 2008), camelina (Peiretti et al., 2007), sage (Peiretti & Meineri, 2008), whole white lupin seeds (Volek & Marounek, 2011), fat-soluble vitamins (Gondret et al., 1998; Dal Bosco et al., 2004) and conjugated linoleic acid (Marounek et al., 2007), results in an increased intake of polyunsaturated FAs. A similar positive correlation between the amount of dietary n-3 PUFA intake and monounsaturated fatty acids (MUFA) content of adipose tissue has been established in lambs (Nürnberg, et al., 1998; Banskalieva et al., 2000), steers (French et al., 2000), pigs (Doichev, 2009) and humans (London et al., 1991).

The described investigations on adipose tissue FA composition in rabbits concern mainly the perirenal fat depot, but no data to other fat depots. This fact was the main motivation for this study, because each of fat depots has not only a different localisation, but also specific metabolic features.

The aim of this study was to identify the differences in the fatty acid composition of subcutaneous and visceral fat depots in healthy New Zealand White rabbits.

MATERIALS AND METHODS

Experimental animals

In this study, 12 (6 male and 6 female) clinically healthy New Zealand White rabbits were used. Rabbits were slaughtered at 90 days of age with an average weight of 3.00±0.03 kg. All animals were fed a pelleted feed twice daily. The ingredients and nutritional composition of pelleted diet is presented in Table 1.
Water was offered *ad libitum*. Rabbits were housed in metal cages with dimensions 80×60×40 cm, at ambient temperature of 20 °C, air humidity 65–70% and 12 h light per day.

The animals were not fasted before being slaughtered. They were slaughtered at a licensed slaughterhouse (Euro Top, Stara Zagora). The study was carried out according to the guidelines of the Animal Ethics Committee at the Faculty of Veterinary Medicine, Trakia University, Bulgaria.

**Adipose tissue sampling**

After removing the skin, feet and paws, the abdominal cavity was opened by incision along the white line (*linea alba*). First, the omental fat depot (OmFD) from each carcass was carefully dissected and collected. Hot carcasses and OmFD samples were chilled at 3–5 °C for 24 hours, then the interscapular (IsFD), inguinal (InFD), pericardial (PeFD) and perirenal (PrFD) fat depots from each carcass were also collected. Obtained samples were well homogenised, packed in polyethylene bags and stored at −20 °C until analysis.

**Gas chromatography**

Fatty acid composition of adipose tissue from each investigated depot was determined after chloroform-methanol extraction of total lipids from 5 g of minced tissue. Chloroform to methanol ratio was 2:1. For methylation of the extract, mixture of methanol and sulfuric acid was used. Methyl esters of fatty acids were separated and quantitated by gas chromatography. Gas chromatography analysis was performed using GS/MS Clarus 500 Gas Chromatograph (PerkinElmer, USA), equipped with flame ionization detector and automatic injection system. The capillary column TG-WAXMS (Thermo Scientific, USA) was filled with polyethylene glycol (PEG) 60 mm × 25 mm × 0.50 μm of size. The carrier gas was hydrogen. The temperature programme was 160 °C for 2 min then increasing at 10 °C/min up to 240 °C where it was maintained for 15 min until completion. Software TotalChrom Tutorial for Version
Fatty acid composition of subcutaneous and visceral fat depots in New Zealand White rabbits

6.3. (PerkinElmer, USA) was used for chromatogram acquisition.

The content of each fatty acid was expressed as a percentage relative to the total amount of fatty acids in depot. The obtained values were used for determination of PUFA/SFA ratios, ratio of omega 6 (18:2+20:2+20:3+20:4) and omega 3 (18:3) unsaturated fatty acids; atherogenic index (AI) and saturation index (SI). For AI and SI, the formulas proposed by Ulbricht & Southgate (1991) were applied:

\[ AI = \frac{(C12:0+4 \times C14:0+C16:0)}{(\sum \text{PUFA}+\sum \text{MUFA})} \]
\[ SI = \frac{(C14:0+C16:0+C18:0)}{(\sum \text{MUFA}+\sum \text{PUFA})} \]

Statistical analysis

All data were presented as mean values and standard deviation (mean ± SD). The statistical processing of data was performed by ANOVA (Statistica v. 6.1, StatSoft Inc., USA, 2002) and statistical significance of differences between groups – by the post hoc LSD test.

Fig. 1. Fat depots in the rabbit carcass. I. Dorsal view of interscapular fat depot. ISD, ISS - right and left lobes; II. Ventral view of inguinal fat depot. IND, INS – right and left parts; III. Ventral view of pericardial fat depot – arrowheads. IV. Ventral view of perirenal fat depot. arrows – right part, asterisks – left part; O – omental fat depot; S – stomach. Bar = 1 cm.
RESULTS

Fig. 1 presents the in situ subcutaneous and visceral fat depots in the rabbit carcass. The results from chromatographic analysis (Table 2) showed that amount of capric (C10:0) and lauric (C12:0) acids were higher in the OmFD than in other depots, but the differences were significant only for lauric acid (P<0.001).

The most significant differences in the content of the myristic acid (C14:0) were reported between the InFD on one hand, and PcFD and PrFD on the other (P<0.001).

The proportion of the myristoleic (C14:1) FA in OmFD differed significantly vs the other depots at P<0.001 (IsFD); P<0.01 (PcFD, PrFD) and P<0.05 (InFD).

The pentadecanoic (C15:0) acid (0.96±0.02%, P<0.001) prevailed in InFD. In all studied fat depots, the percent of the palmitic acid (C16:0) was high. The differences with the highest statistical significance were established between InFD vs both PcFD and PrFD (P<0.001).

Table 2. Fatty acid composition of fat depots in New Zealand White rabbits, fed a standard diet. Data are presented as mean ± SD, n=12

<table>
<thead>
<tr>
<th>Fatty acids, %</th>
<th>IsFD</th>
<th>InFD</th>
<th>PcFD</th>
<th>PrFD</th>
<th>OmFD</th>
</tr>
</thead>
<tbody>
<tr>
<td>C10:0, capric</td>
<td>0.08±0.03</td>
<td>0.15±0.03</td>
<td>0.10±0.01</td>
<td>0.18±0.04</td>
<td></td>
</tr>
<tr>
<td>C12:0, lauric</td>
<td>0.31±0.01</td>
<td>0.33±0.01</td>
<td>0.16±0.02</td>
<td>0.51±0.02</td>
<td></td>
</tr>
<tr>
<td>C14:0, myristic</td>
<td>4.49±0.04</td>
<td>5.43±0.88</td>
<td>2.92±0.09</td>
<td>5.38±0.10</td>
<td></td>
</tr>
<tr>
<td>C14:1 Δ⁹ (n-5), myristoleic</td>
<td>0.22±0.01</td>
<td>0.27±0.03</td>
<td>0.25±0.02</td>
<td>0.31±0.03</td>
<td></td>
</tr>
<tr>
<td>C15:0, pentadecanoic</td>
<td>0.89±0.04</td>
<td>0.96±0.02</td>
<td>0.52±0.03</td>
<td>0.86±0.10</td>
<td></td>
</tr>
<tr>
<td>C16:0, palmitic</td>
<td>38.30±0.73</td>
<td>41.05±1.8</td>
<td>33.26±0.61</td>
<td>37.12±0.32</td>
<td></td>
</tr>
<tr>
<td>C16:1 Δ⁹ (n-7), palmitoleic</td>
<td>4.65±0.17</td>
<td>3.60±0.02</td>
<td>4.78±0.04</td>
<td>4.67±0.09</td>
<td></td>
</tr>
<tr>
<td>C17:0, margaric</td>
<td>0.60±0.01</td>
<td>0.67±0.02</td>
<td>0.69±0.01</td>
<td>0.76±0.11</td>
<td></td>
</tr>
<tr>
<td>C18:0, stearic</td>
<td>5.17±0.07</td>
<td>5.11±0.34</td>
<td>2.44±0.04</td>
<td>2.90±0.49</td>
<td></td>
</tr>
<tr>
<td>C18:1 Δ⁹ (n-9), oleic</td>
<td>9.05±0.15</td>
<td>7.02±0.06</td>
<td>6.48±0.05</td>
<td>6.06±0.05</td>
<td></td>
</tr>
<tr>
<td>C18:2 Δ⁹,¹² (n-6), linoleic</td>
<td>32.13±0.77</td>
<td>31.17±1.3</td>
<td>42.77±0.10</td>
<td>44.26±0.96</td>
<td>36.51±0.04</td>
</tr>
<tr>
<td>C18:3 Δ⁹,¹²,¹⁵ (n-3), α-linolenic</td>
<td>2.90±0.03</td>
<td>3.08±0.03</td>
<td>3.17±0.03</td>
<td>3.50±0.29</td>
<td></td>
</tr>
<tr>
<td>C20:0, arachidic</td>
<td>0.16±0.01</td>
<td>0.18±0.02</td>
<td>0.14±0.01</td>
<td>0.10±0.01</td>
<td></td>
</tr>
<tr>
<td>C20:1 (n-9, Δ¹¹), eicosenoic</td>
<td>0.39±0.02</td>
<td>0.40±0.04</td>
<td>0.24±0.03</td>
<td>0.26±0.01</td>
<td></td>
</tr>
<tr>
<td>C20:2 (n-6, Δ¹¹,¹⁴), eicosadienoic</td>
<td>0.32±0.01</td>
<td>0.29±0.01</td>
<td>0.3±0.01</td>
<td>0.27±0.02</td>
<td></td>
</tr>
<tr>
<td>C20:3 (n-6, Δ¹¹,¹⁴), eicosatrienoic</td>
<td>0.06±0.01</td>
<td>0.03±0.01</td>
<td>0.06±0.01</td>
<td>0.41±0.01</td>
<td></td>
</tr>
<tr>
<td>C20:4 (n-6, Δ¹⁸,¹¹,¹⁴), arachidonic</td>
<td>0.28±0.01</td>
<td>0.26±0.03</td>
<td>0.23±0.01</td>
<td>0.20±0.01</td>
<td></td>
</tr>
</tbody>
</table>

IsFD – interscapular fat depot; InFD – inguinal fat depot; PcFD – pericardial fat depot; PrFD – perirenal fat depot; OmFD – omental fat depot.
Palmitoleic acid (C16:1) content in the pericardial fat depot was 10.63±2.60%, while in the interscapular, perirenal, omental and inguinal FD, its content was almost twice lower (P<0.001).

The margaric acid (C17:0) content was the highest in the omental (0.76±0.11%) than in other fat depots, but significant differences were observed only between OmFD and IsFD (P<0.01), as well as OmFD and PcFD (P<0.05).

There were no statistically significant differences between the percentage of stearic acid (C18:0) in IsFD (5.17±0.07%), InFD (5.11±0.34%) and PcFD (5.03±0.04%). The lowest percentage of this acid was observed in the PrFD (2.44±0.04%, P<0.001).

Oleic acid (C18:1) prevailed in the IsFD (9.05±0.15%). There were statistically significant differences between the content of this fatty acid in IsFD vs both OmFD and PrFD (P<0.001); InFD (P<0.001); PcFD (P=0.05)

In all tested rabbit fat depots, a high content of linoleic acid (C18:2) was established: 44.26±0.96% in the perirenal and 42.77±1.19% in the pericardial depot. No considerable differences were demonstrated between linoleic acid percentages in subcutaneous depots as well as between PcFD and PrFD. The content of this FA in OmFD was the lowest and differences with other depots were significant (P<0.001).

In the omental depot, α-linolenic acid (C18:3) content was higher than in other fat depots, although significant differences were found only between OmFD and IsFD (P<0.05).

There were no differences between the values of arachidic (C20:0) FA in IsFD, InFD and PcFD but these were established (P<0.001) between abovementioned depots and OmFD.

The percentages of the eicosenoic (C20:1) acid in IsFD, InFD and PcFD were significantly higher than in PrFD (P<0.001). The eicosadienoic (C20:2) FA prevailed in interscapular fat and exhi-
bited statistically significant differences vs OmFD (P<0.001); InFD and PcFD (P<0.01); PrFD (P<0.05). The highest level of eicosatrienoic acid (C20:3) was established in the omental fat (P<0.001). Arachidonic (C20:4) acid contents in visceral depots were similar (Table 2).

In the IsFD, percentages of the saturated (SFA) and unsaturated fatty acids (USFA) were equal (Table 3). In the other subcutaneous depot – InFD, SFA content was 1.17 times higher than that of USFA.

InFD had the highest atherogenic index and saturation index and the lowest n-6/n-3 ratio (P<0.001). In all investigated visceral depots, USFA were prevalent and PUFA/SFA ratio – the highest. The lowest atherogenic and saturation indices were demonstrated in the perirenal depot (P<0.001), but the highest PUFA/SFA ratio was calculated for the perirenal depot (P<0.001).

Indices related to human health are presented in Table 3.

DISCUSSION

One of the most popular rabbit breeds for meat production is the New Zealand White. Lately, the New Zealand rabbit is a preferred animal model, widely used to study visceral obesity, atherosclerosis (Yanni, 2004), metabolic syndrome and insulin resistance (Mitsuguchi et al., 2008; Zhao et al., 2008; Georgiev et al., 2011; Ivanova et al., 2015; Niimi et al., 2016). In rabbits, not only the visceral, but also the subcutaneous fat is formed in depots (Cinti, 2005). Subcutaneous and visceral fat deposits in the rabbit differ substantially both structurally and metabolically (Yonkova et al., 2014). The results of the present study convincingly confirm these findings and show that each fat depot is characterised by specific fatty acid profile.

According to the criteria of Blasco & Ouhayoun (1993), there is a fat depot to each body part, intended for consumption. The relevant part of the interscapular depot remains to the fore limbs, the pericardial fat – to the thoracic cage, the perirenal fat – to the loin and the inguinal fat depot – to the hind limbs. From the established differences in fatty acid composition between subcutaneous and visceral depots, it can be assumed that the different parts of the rabbit carcass have different atherogenic indices, which is important for the human nutrition. In rabbits fed different supplements rich in n-3 fatty acids (Cobos et al., 1993; Peiretti et al., 2007; Peiretti & Meineri, 2008) and reared under various production systems (Lazzaroni et al., 2009), only changes in fatty acid composition of the perirenal depot have been established. This is understandable, because its mass is essential for the fat content of the rabbit carcass (Blasco & Ouhayoun, 1993). The palmitic acid percentage in the perirenal fat was too close to values measured by Lazzaroni et al. (2009) in rabbits from the Carmagnola Grey breed.

Our studies showed that in the PrFD, linoleic acid predominated over palmitic acid content. This is in contrast to Peiretti et al. (2007) and Lazzaroni et al. (2009), which found more palmitic acid in the same depot. The values of oleic, linoleic and palmitic acid in this study are also different from results obtained by Marounek et al. (2007) for Hyplus rabbits.

Therefore, the fatty acid composition of the perirenal depot depends not only on nutrition, but is significantly affected by the breed, age and way of rearing of the rabbits.
The fatty acid profile of the other subcutaneous depot – the interscapular and the inguinal, were not investigated by the aforementioned authors. Tables 2 and 3 show that SFA content was the highest (53.88±3.12%, P<0.001) in the inguinal depot compared to all other depots. From the SFA, palmitic and myristic percentages were the highest, followed by pentadecylic and arachidonic FAs. As a consequence, the depot has the highest AI and SI. These results are in line with the statement of Ulbricht & Southgate (1991) that myristic and palmitic fatty acids are atherogenic and hyperlipidaemic.

The fatty acid profile of the other subcutaneous depot – the interscapular is rather different. Although the proportions of palmitic (38.3±0.73%) and stearic (5.17±0.07%) FA was high, the depot had the highest levels of long-chain unsaturated FAs – oleic (9.05±0.15%), eicosa-dienoic (0.32±0.01%) and arachidonic (0.28±0.01%). Therefore, the ratio between MUFA and USFA in interscapular depot was 1:1 and AI and SI values were lower, compared with those of the inguinal depot. In the subcutaneous fat depots in rabbits, oleic acid content was 5 times lower, while linoleic acid content – 5 times higher, than values reported by Doichev (2009) in tallow from pigs.

In visceral fat depots, the highest percentage of USFA was observed in the pericardial depot (65.63±0.39%), which is crucial for the lowest levels of atherogenic (0.60) and saturation (0.50) indices. Among intraabdominal depots, statistically significantly higher USFA share was demonstrated in the perirenal depot (59.77±1.16%), compared with the omental depot (52.19±0.55%). So, retroperitoneal perirenal fat is characterised by lower AI and SI, in comparison with respective values of indices in the intraperitoneal omental fat.

Therefore, specific fatty acid profiles of each fat depot should be taken into account when using the rabbit as an animal model of central obesity (Caroll et al., 1996; Zhao et al., 2008; Georgiev et al., 2011; Ivanova et al., 2015; Niimi et al., 2016). For this reason, the omental depot was included in this study. It is not associated to the dietary properties of rabbit meat, but is of interest to the investigations on abdominal obesity (Shen et al., 2003). Unlike the perirenal depot, which drainage is carried out by the caudal vena cava, the drainage of the omental fat is performed by the portal vein. The free fatty acids released from the omental depot directly reach and affect the liver, hence the theory of visceral obesity based on the "portal hypothesis" (Huffman & Barzilai, 2009; Tran & Kahn, 2010).

In conclusion, the anatomical localisation of fat depots in rabbits is critical not only for their structure, but also for their fatty acid composition, which proved the specific metabolism in each of them. In all fat depots, palmitic, linoleic and oleic fatty acids were predominant. Therefore, both the meat and the fat of rabbits are of high biological and nutritional value due to the beneficial fatty acid profile from human health point of view.

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