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TECHNICAL NOTE

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Characterization of the Triacylglycerol Crystal Formation in Adipose Tissue During a Vehicle Collision

ABSTRACT: The unusual appearance of crystalline fat structures was observed during the postmortem examination of a motor vehicle accident victim. The crystal structures were characterized using Fourier transform infrared spectroscopy and x-ray diffractometry. The structures were found to be made of triacylglycerols, a dominant lipid structure found in human adipose tissue, capable of forming various polymorphic structures. The morphology of the crystalline material was found using both techniques to be predominantly the β′ form of triacylglycerols. The accelerated growth of such triacylglycerol morphology has been observed as a result of shear stresses in other studies involving edible fats. As a result of the findings of this study, it is proposed that increased shear forces may be responsible for the formation of the unusual fat structure found in the victim. An understanding of the effect of forces on the structure of body fat in high-impact collisions can potentially assist in verifying a high-velocity impact.

KEYWORDS: forensic science, triacylglycerols, adipose, crystal, vehicle accident, infrared spectroscopy, x-ray diffractometry

The formation of agglomerations of crystalline fat structures has been observed in a pathology sample obtained from the adipose tissue of the victim of a motor vehicle accident. As the formation of such a crystal structure in fat not commonly observed in postmortem examinations, a study has been carried out to characterize the material in order to understand the origin.

Human adipose tissue is made up of mainly lipids (greater than 95%) and the majority of the lipids are triacylglycerols (1). The precise fatty acid composition of human triacylglycerols will depend on factors such as diet and gender. However, there are fatty acids known to be more commonly observed in human adipose tissue. Oleic acid (45–50%) and palmitic acid (20–30%) are the major fatty acids found, with linoleic, stearic, and palmitoleic acids also present and others in trace quantities.

Triacylglycerol molecules can form various polymorphic forms (2,3). The most common forms are termed α, β′ and β in order of increasing melting point, packing density and stability. The packing modes of the polymorphs of triacylglycerols have been widely reported (4–6). The α form shows a hexagonal subcell, the β′ form shows an orthorhombic perpendicular subcell while the β form displays a triclinic parallel subcell. The α form is the least stable and easily transforms to either the β′ form or the β form. The crystallization of triacylglycerols depends on various factors including the composition, the thermal history and the shear rates applied. It is also possible for the crystal structures of triacylglycerols to form clusters or polycrystals (7).

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In the present study, the nature of the lipid crystal structure formed in the adipose tissue collected from the postmortem examination of the victim of a vehicle accident is examined using Fourier transform infrared (FTIR) spectroscopy and x-ray diffractometry (XRD). FTIR spectroscopy enables the molecular conformation of triacylglycerol molecules to be examined and the crystal packing to be characterized. XRD also provides a useful method for characterizing the crystalline structures present. The unusual nature of the crystalline structures present is potentially related to the shear effects of a high speed collision. Analysis of force induced changes in fat could assist with accident reconstruction in events such as air craft crashes or explosions by allowing assessment of the forces experienced by individual bodies or body parts. It could also be utilized to verify or refute accounts of motor vehicle collisions with respect to velocity at the time of impact.

Methods

Pathology

The deceased was a 26-year-old male driver from a motor vehicle collision. The car had left the road and collided with a tree following an over-taking maneuver. The impact caused the vehicle to split into three sections and come to rest 20 m from the tree. The victim was declared deceased at the scene and transported to the mortuary where the body was stored at 4°C until the postmortem examination 3 days later.

FTIR Spectroscopy

The infrared spectra were recorded using a Nicolet Magna-IR 760 FTIR spectrometer coupled to a Nic-Plan infrared microscope with a liquid N_2 cooled mercury cadmium telluride detector. Samples were placed on a diamond window and analyzed in the microscope using the transmission mode. For each spectrum, 512 scans were collected from 4000 to 650 cm^{-1} at a resolution of 2 cm^{-1}.

XRD

Analysis of the crystalline phases was carried out by a Siemens D5000 X-ray Powder Diffractometer fitted with a graphite post-monochromator. An x-ray tube was used at a power of 40 kV and 30 mA to produce Cu-Kα radiation. The scanning was conducted in the 2θ range of 10° and 28° with 1° and 0.2 mm divergence and receiving slits, respectively. The sample was mounted on a low background quartz plate on which it was allowed to spread and dry over a period of 7 days at ambient temperature.

Results

Pathology

At the postmortem examination it was noted the body was almost bloodless so subdural blood had to be collected for toxicological analysis as none was obtainable from the peripheries. No free blood was seen in the body cavities (which were not breached) despite the presence of complete aortic transection, liver lacerations, avulsion of the spleen, and pelvic disruption. The body exhibited no macroscopic evidence of decomposition and routine microscopic examination of tissue samples revealed no evidence of autolysis.

The presence of crystalline-appearing fat was noted in the chest, abdominal and pelvic cavities. The largest collection was found in the left ventricle of the heart (Fig. 1), which contained no blood when it was opened. The pale white/cream fat crystals measured approximately 1–2 mm across, with the single largest localized cluster of crystals measuring 8 × 10 × 5 mm. During collection it was noted that normal body fat was liquidizing at room temperature, but the crystalline region of fat maintained its morphology. A fat sample containing a mixture of crystalline and noncrystalline regions was collected and stored at 4°C prior to analysis. Death was attributed to multiple injuries. Toxicological analysis for alcohol and common drugs was negative.

FTIR Spectroscopy

FTIR microscopy was used to record the infrared spectrum of the crystalline region of the sample, as well as a spectrum for a liquid region of the sample. The spectra are shown in Figs. 2 and 3 and show characteristic bands for triacylglycerols. However, there are significant differences in the spectra and these may be attributed to the different polymorphic structures formed by the triacylglycerol molecules in the fat. Bands associated with the methylene chains of the triacylglycerols can be attributed to the presence of the β' form of the polymorph for the crystalline region of the sample, while the liquid regions studied reveal an α polymorph.

FIG. 1—*Heart opened to show mitral valve. Arrows illustrate the crystalline deposits on the endocardial surface of the heart and between the chordae of the mitral valve.*

The CH_2 scissoring band for the liquid region appears at 1467 cm^{-1}, while a doublet with peaks at 1473 and 1465 cm^{-1} is observed for the crystalline region. The single band at 1467 cm^{-1} has been assigned to an α polymorph for various triacylglycerols (8). The splitting of the CH_2 scissoring band into two components is due to intermolecular interaction between chains and reflects a crystal with an orthorhombic cell of a β' polymorph (8–11). The CH_2 rocking band is similarly affected for the crystalline region: a doublet with peaks at 720 and 729 cm^{-1} is observed. A single band at 723 cm^{-1} is observed for the liquid region. It has been reported that the α form does give rise to a single CH_2 rocking band near 720 cm^{-1} due to the hexagonal packing of the hydrocarbon chains (11). Other bands associated with the CH_2 chains in the triacylglycerols, such as the C-H stretching bands in the 3000–2800 cm^{-1} range, also show changes due to different packing arrangements.

Major differences are also observed to the C=O stretching bands in the 1780–1710 cm^{-1} region of the spectra. The liquid sample studied shows a broad band centered at 1747 cm^{-1}, while the crystalline sample shows two distinct peaks at 1741 and 1727 cm^{-1}. The latter two peaks have been assigned to a β' form in a study of 1,2-dipalmitoyl-3-myristoyl-sn-glycerol (10). The C=O stretching bands are sensitive to the glycerol conformation. It is well established that the bending and packing of chains affects the position of the carbonyl band.

XRD

The diffractogram of the fat sample is illustrated in Figure 4. The d-spacing values corresponding to the 2θ values of the main peaks are listed in Table 1. The assignments are based on comparison with other XRD studies of triacylglycerols (3,12–15). The positions of the XRD peaks indicate that the dominant structure present in a β' polymorph. There is also a peak at 4.53 Å ($2\theta = 19.4°$) which may be attributed to the β polymorph. However, when β polymorph is a dominant structure this peak is observed to be strong, indicating that any β polymorph is present in small quantities in the current sample. The presence of the α form is not confirmed by the XRD data, but as this form shows a broad peak at about 4.2 Å there could be overlap with a β' peak. The assignment of the peak at 5.41 Å is more difficult to assign and was not observed in similar XRD analyses by Szydlowska-Czerniak and co-workers (12). However, it is most likely to be β' as it matches closely with the calculated indexing of 5.43 Å peak belonging to the orthorhombic β' polymorph (16).

Discussion

Examination of the crystal structure formed in the abnormal postmortem sample using FTIR spectroscopy and XRD has revealed the presence of a β' polymorph structure of the triacylglycerols present. Significantly, the crystal structures were observed in clusters. Stable β' crystallites are known to form clusters of spherulites or polycrystals of 10–60 μm in size and it has been

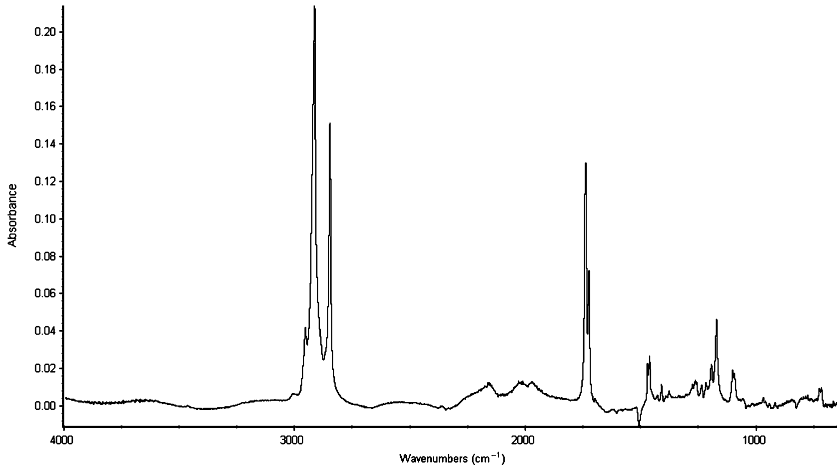

FIG. 2—*FTIR spectrum of crystalline region of fat sample.*

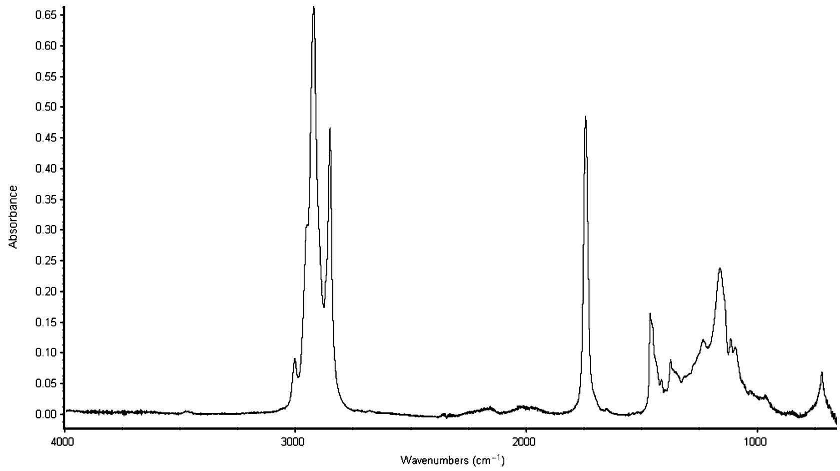

FIG. 3—*FTIR spectrum of liquid region of fat sample.*

observed that this sort of spherulite may agglomerate into flocs of up to 600 μm in size (7,17). The occurrence of such clusters is not usually observed postmortem in human adipose tissue. However, this type of β′ polymorph structure has been observed in the food industry, where triacylglycerols are commonly encountered in edible fats and oils.

The growth of crystal structures depends on the temperature to which the fat is heated, the annealing time and the cooling rates. For the current sample, it is possible that a temperature increase during impact could be responsible for nucleation and the subsequent cooling of the body results in the formation of crystal structures. However, the appearance of agglomerates of crystals indicates an acceleration of the crystallization process. The possible nucleation time of the vehicle impact does not necessarily explain the degree of crystallization.

Another important factor affecting the resulting crystal structure of fats is shear. The effect of shear on triacylglycerol crystal growth has been examined in a number of studies of edible oils and fats (7,18–20). Shear is used in industrial processes to crystallize fats while mixing and varying the shear rate has been demonstrated to affect the crystallization process. For the sample in the current study, the acceleration of crystal growth may be due to a high shear rate occurring during the vehicle impact. It is proposed that the region of the adipose tissue where the crystal clusters were observed could have undergone considerable shear forces during deceleration of the victim within the vehicle.

An increase in shear rate has been reported to accelerate the formation of β′ crystal formation in palm oil triacylglycerols (7). The formation of agglomerated β′ crystal structures were also observed for that study. Variation of shear rates showed that the phase transition from an α phase to a β′ phase was accelerated by increasing shear. The β′ phase nucleates on the α crystallites and the amount of β′ crystal formation depends on the rate of transformation of α to β′ as well as the rate that β′ grows from the melt. The acceleration effect for palm oil was explained by the increased α-β′ transition under shear. At high shear the α nuclei form many separate small crystallites which can easily transform into β′, but at lower shear rates the α nuclei can agglomerate and so retard the nucleation of β′ crystals.

Conclusions

FTIR spectroscopy and XRD were used to characterize the unusual fat crystals observed in the postmortem adipose tissue of the victim of a vehicle accident involving a high speed impact. The

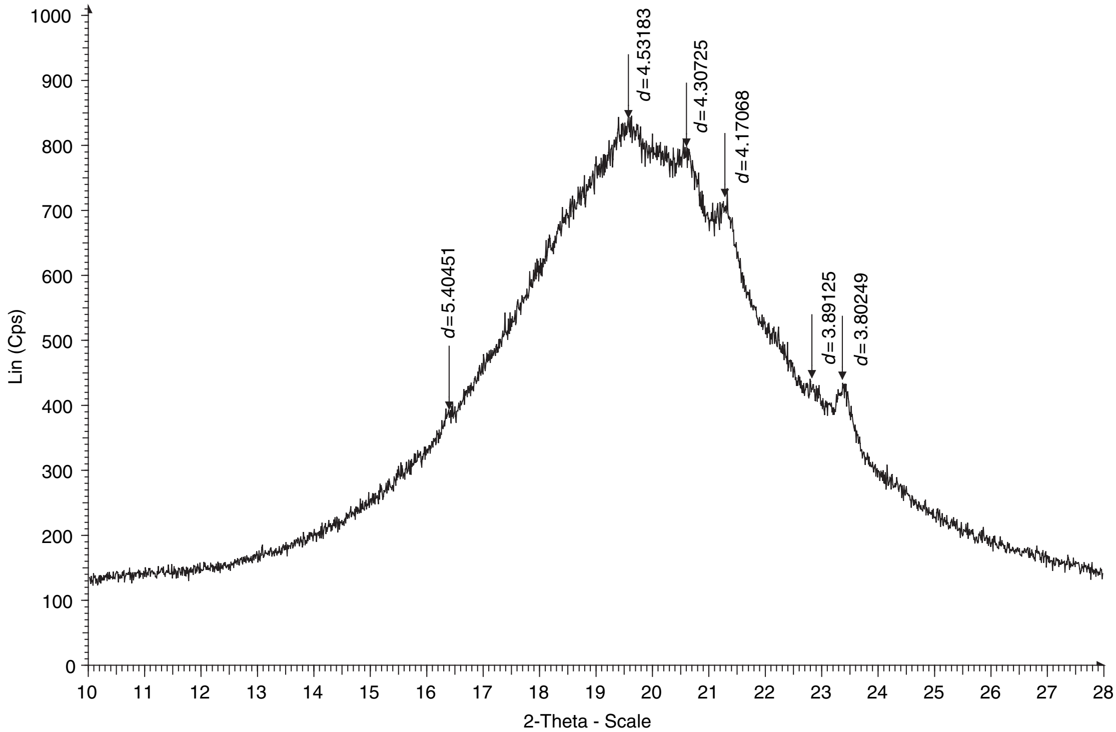

FIG. 4—*Diffractogram of fat sample.*

TABLE 1—*XRD analysis of fat sample.*

d-spacing/Å	Polymorph
5.41	Uncertain (most likely to be β′)
4.53	β
4.31	β′
4.17	β′
3.89	β′
3.80	β′

agglomerated crystals were found to consist of β′ polymorphs of triacylglycerols. It is proposed that the accelerated formation of such crystals may be due to a high shear rate experienced by the adipose tissue during the deceleration of the vehicle during the accident.

Further studies involving the effect of shear rate on model lipid systems will be used to investigate the mechanism of crystal formation. Such studies should provide some valuable information about the forces involved in particular vehicle impacts and will be used to confirm whether or not shear forces are responsible for the formation of crystal clusters. If abnormal crystal structures are only formed as a result of high shear their study may provide information regarding the degree of acceleration or deceleration that occurred during a collision and assist with incident reconstruction.

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