MODIFICATIONS IN THE STRUCTURE OF THE TRIACYLGLYCEROLS OF BOVINE MILK FAT

A thesis presented in partial fulfilment of the requirements for the degree of Doctor of Philosophy in Biochemistry at Massey University

Ian Malcolm Morrison

1976

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ABSTRACT

A pair of monozygous twin cows was used to investigate the influence of the increased availability of linoleic acid (18:2) to the mammary gland, on the structure and physical properties of the milk triacylglycerols (TGs). The cows were grazing fresh pasture, and in addition one of the pair was provided with a daily supplement of encapsulated sunflower oil. The milk fat of the cow given the supplement (18:2-rich milk fat) contained 15.5% 18:2 compared with 1.8% 18:2 in the milk fat of the other cow (control milk fat). This increase in the proportion of 18:2 in the milk fat was accompanied by decreases in the proportions of myristic acid (14:0) and palmitic acid (16:0). The effect of this altered fatty acid (FA) composition on the TG composition of the 18:2-rich milk fat was to increase the proportions of TGs with 40, 52 and 54 acyl carbons and to decrease the proportions of TGs with 34, 36, 38. 44, 46, 48 and 50 acyl carbons relative to the proportions in the control milk fat.

Both milk fat samples were separated, by column chromatography on silicic acid, into TG fractions of high, medium and low molecular weight. The relative proportions of the TG fractions of high, medium and low mol. wt. in the 18:2-rich milk fat were 43.0, 19.5 and 37.5% respectively compared with 36.1, 19.7 and 44.2% respectively in the control milk fat. Stereospecific analysis of these triacylglycerol fractions demonstrated that in fractions of similar mol. wt., the distribution of fatty acids within the TG molecule in the presence of high levels of 18:2, was not appreciably altered from that in the triacylglycerol fractions of the control milk fat. The positional distribution of fatty acids in the triacylglycerols of the total milk fats was also similar. In the milk fat, containing high levels of

linoleic acid, 18:2 showed a preference for positions 1 and 2 in the triacylglycerols of the low and medium mol. wt. fractions, and for positions 2 and 3 of the high mol. wt. fraction.

The three TG fractions of each milk fat were further resolved into TG classes of differing levels of unsaturation. The 18:2-rich milk fat contained higher levels of the unsaturated TGs, namely the diene, triene and tetraene TGs, and lower levels of the saturated TGs and to a lesser extent the monoene TGs. The diene TGs of the 18:2-rich milk fat included combinations of 18:2 with two saturated FAs, which are a minor constituent of normal milk fats. Likewise the triene TGs reflected the presence of 18:2 in combination with 18:1 and a saturated FA.

The thermal properties of the two milk fats were examined to investigate the influence of the altered TG composition and structure on the physical characteristics of the milk fat. The 18:2-rich milk fat melted at a lower temperature than the control milk fat with a large proportion of the sample melting over the narrow temperature range between 5 and 15^{o}C. In contrast the control milk fat melted over a much wider temperature range.

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ABBREVIATIONS

ATP	adenosine-5′-triphosphate
B	butyric acid
BSA	bovine serum albumen
CoA	coenzyme A
cpm	counts per minute
DG	diacylglycerol
dpm	disintegrations per minute
D.S.C.	differential scanning calorimeter
D.T.A.	differential thermal analysis
DTT	dithiothreitol
EDTA	ethylenediaminetetraacetic acid
Eth.	ethanol
FA	fatty acid
Fw	weight response factor
g.l.c.	gas-liquid chromatography
HDL	high density lipoprotein
i.d.	internal diameter
LDL	low density lipoprotein
M	moles per litre
MG	monoacylglycerol
nmoles	nanomoles
N.M.R.	nuclear magnetic resonance
O	oleic acid
Orig.	original
P	palmitic acid
P-Ph	phosphoryl phenol
2,3-PL	2,3-diacyl-sn-glycerol-3-phosphoryl phenol
1-PL	1-acyl-sn-glycerol-3-phosphoryl phenol
R_f	distance moved relative to solvent front
s	seconds
St	stearic acid
TG	triacylglycerol
t.l.c.	thin-layer chromatography
Tris	tris(hydroxymethyl)aminomethane
VLDL	very low density lipoprotein

ABBREVIATIONS

Triacylglycerols of milk fat are referred to by their total number of acyl carbon atoms e.g. Glycerol tristearate = C_{54}.

Pure stereospecific and racemic triacylglycerols are abbreviated as by Litchfield (1972) e.g. sn-glycerol-1-palmitate-2-oleate-3-stearate = sn - POSt.

Fatty acids are designated by the shorthand notation - number of carbon atoms : number of double bonds, e.g. octadecanoic acid (stearic acid) = 18:0.

Shorthand notation for triacylglycerols separated by degree of unsaturation is written as 000 = saturated TG, 001 = monounsaturated or monoene TG (where 0 and 1 refer to saturated and monoenoic fatty acids respectively). When the stereospecific distribution of fatty acids in the triacylglycerols is known, these triacylglycerols are referred to by double underlining e.g. 020 = diene TG containing a dienoic fatty acid in position 2.

Other abbreviations used in this thesis, unless otherwise designated in the text, follow the guide lines set down by the Biochemical Journal (1973) 131, 1 - 20.