ABSTRACT

The main objective of present study is to establish the relationship between positional fatty acids in triacylglycerols and obesity. The inferences drawn from the *in vivo* studies have been used in the design of prospective structured lipids to reduce obesity risk. In the effort to evaluate the effect of positional fatty acids on obesity, an accurate and precise analytical method to analyse positional distribution of fatty acids within the glycerol moiety is an important prerequisite. An elegant, user-friendly and accurate analytical method using quantitative ^{13}C NMR spectroscopy (qCNMR) has been fully established in the present study. A regiospecific analysis data using qCNMR was attainable within 44 minutes, much shorter relative to the conventional methods which use a combination of chromatographic techniques. Besides, the current method provides quantitative results with high accuracy by virtue of direct measurement and negligible sample preparation prior to the analysis. Occurrence of acyl migration during the course of sample pre-treatment is omitted. The applicability as well as the versatility of the present method had been accessed in various oils and fats, reaction intermediates of chemical interesterification and extracted lipids from biological samples with systematic errors were less than 2.0 mol%. As a summary, a cookbook approach for qCNMR in the regiospecific analysis of oils and fats from diverse sources has been established.

Correlation between the positional fatty acids within glycerol moiety and the accretion of fat was investigated in two *in vivo* studies using C57BL/6 mouse model. The positional distribution of saturated fatty acid (SFA) was found to exert a more pronounced effect on body fat deposition than the total SFA content. The extent of fat deposition will be lessened in the event of long chain SFA (C16:0 and above) occur predominantly at the *sn*-1,3 positions of triacylglycerols. Among the different chain

length of SFA, stearic acid (C18:0) was found to be more efficient in reducing fat deposition than palmitic acid (C16:0) at the similar positions. It is postulated that after the action of 1,3-specific pancreatic lipase, non-esterified SFA will suffer delayed absorption and being excreted in the faeces due to the formation of insoluble calcium soaps. Consequently, the resynthesis of triacylglycerols in chylomicron and eventually their deposition in adipose tissues will be reduced.

In accordance with the findings from both *in vivo* studies, structured lipid which aims to alleviate the risk of obesity has been designed. An even longer SFA, namely behenic acid (C22:0), was incorporated into the *sn*-1,3 positions of triacylglycerols in palm olein iodine value (IV) 56 and high-oleic sunflower oil. To achieve sustainable chemistry, immobilised lipases from the strain of *Rhizomucor miehei* (Lipozyme RM IM, Novozymes^{TM}) and *Thermomyces lanuginosa* (Lipozyme TL IM, Novozymes^{TM}) were employed in the current synthesis work. The synthesized structured lipids which contain high amount of BOO and BOB molecular species may serve potential applications in functional dietary fats, for instance, bakery shortening, *trans*-fat-free margarine, vanaspati and cocoa butter equivalent, with the additional health benefit in terms of giving lower risk towards the body fat deposition.

ABSTRAK

Objektif utama kajian ini adalah untuk menentukan hubungan antara kedudukan asid lemak dalam triasilgliserol dengan obesiti. Kesimpulan yang didapati daripada kajian *in vivo* telah digunakan dalam reka bentuk dan sintesis lipid berstruktur yang bertujuan untuk mengurangkan risiko obesiti. Dalam usaha mengkaji kesan kedudukan asid lemak dalam obesiti, satu kaedah analisa yang tepat adalah penting untuk menganalisa komposisi asid lemak pada kedudukan yang berbeza dalam triasilgliserol. Satu kaedah analisa yang elegan, mudah dan tepat telah dioptimakan sepenuhnya dengan menggunakan kuantitatif spektroskopi ^{13}C NMR (qCNMR). Data analisa regiospesifik dapat diperoleh dalam 44 minit dengan menggunakan qCNMR. Ini memerlukan masa yang lebih pendek daripada kaedah konvensional yang menggabungkan teknik-teknik kromatografi. Selain itu, kaedah ini memberi keputusan kuantitatif yang lebih tepat kerana penyediaan sampel tidak diperlukan sebelum analisa. Fenomena pemindahan asil semasa penyediaan sampel dapat dielakkan. Kesesuaian serta keserbabolehan kaedah ini telah diuji dalam pelbagai jenis minyak dan lemak, antaranya produk interesterifikasi kimia dan ekstrak lipid daripada sampel biologi dengan ralat sistematik yang kurang daripada 2.0 mol%. Kesimpulannya, protokol qCNMR dalam analisa regiospesifik minyak dan lemak telah dioptimumkan.

Hubungan antara kedudukan asid lemak dalam triasilgliserol dan pemendapan lemak badan telah dikaji dalam dua kajian *in vivo* yang menggunakan model tikus C57BL/6. Kedudukan asid lemak tepu (SFA) didapati memberi kesan yang lebih ketara atas penyerapan lemak badan jika dibandingkan dengan kandungan SFA keseluruhan. Penyerapan lemak badan dapat dikurangkan sekiranya kebanyakan SFA berantai panjang (C16:0 dan ke atas) berada di kedudukan *sn*-1,3 dalam triasilgliserol. Antara

panjang rantai SFA yang berbeza, asid stearik (C18:0) didapati lebih berkesan dalam mengurangkan penyerapan lemak daripada asid palmitik (C16:0) pada kedudukan yang sama. Ini adalah kerana selepas tindakan pankreas *lipase* yang bertindak khusus pada kedudukan *sn*-1,3, SFA yang bebas akan mengalami penangguhan dalam penyerapan dan akhirnya akan dikeluarkan dalam najis sebab pembentukan sabun kalsium tidak terlarut. Oleh itu, resynthesis triasilgliserol di *chylomicron* dan akhirnya penyerapan lemak dalam tisu-tisu adipos akan dikurangkan.

Selaras dengan penemuan dari kedua-dua kajian *in vivo*, lipid berstruktur yang bertujuan untuk mengurangkan risiko obesiti telah direka dan disintesis. Rantai SFA yang lagi panjang, iaitu asid behenik (C22:0), telah digabungkan pada kedudukan *sn*-1,3 dalam triasilgliserol yang diperolehi daripada olein sawit bernilai iodin (IV) 56 dan minyak bunga matahari yang beroleik tinggi. Untuk mencapai kemampanan, *lipases* semulajadi *Rhizomucor miehei* (Lipozyme RM IM, Novozymes^{TM}) dan *Thermomyces lanuginosa* (Lipozyme TL IM, Novozymes^{TM}) telah digunakan dalam kerja-kerja sintesis. Lipid berstruktur yang disintesiskan mengandungi komposisi BOO dan BOB spesies yang tinggi. Ini membolehkan aplikasi-aplikasi dalam pemakanan lemak berfungsi, sebagai contoh, roti lemak sayuran, marjerin yang bebas dari *trans*-asid lemak, vanaspati dan kesamaan lemak koko, dengan manfaat kesihatan dari segi memberi risiko yang lebih rendah terhadap penyerapan lemak badan.

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%	per cent
ACQTM	acquisition time
ACUC	Animal Care and Use Committee
ANOVA	analysis of variance
AOCS	American Oil Chemists' Society
BBB	tribehenoylglycerol
BBP	1,2-dibehenoyl-3-palmitoylglycerol
BBS	1,2-dibehenoyl-3-stearoylglycerol
Bhd.	Berhad (limited)
BOB	1,3-dibehenoyl-2-oleoylglycerol
BOO	1-behenoyl-2,3-dioleoylglycerol
BOSt	1-behenoyl-2-oleoyl-3-stearoylglycerol
BSTFA	N,N-Bis(trimethylsilyl)trifluoroacetamide
°C	degree Celsius
^{13}C	carbon-13
Ca	calcium
$CDCl_3$	deuterated chloroform
CLA	conjugated linoleic acid
CMC	critical micelle concentration
CN	carbon number of the three acyl chains
COB	cocoa butter
$Cr(acac)_3$	chromium(III) acetylacetonate
DB	number of double bonds
DHA	docosahexaenoic acid
DNPU	dinitrophenyl urethane
DSC	differential scanning calorimetry
ECN	equivalent carbon number
ELSD	evaporative light scattering detector
EPA	eicosapentaenoic acid
etc.	et cetera
FA	fatty acid
FAC	fatty acid composition

FID	free induction decay
GC	gas chromatography
G(x)	Gaussian function
^{1}H	proton
HDL-C	high-density-lipoprotein cholesterol
HOS	high-oleic sunflower oil
HPLC	high performance liquid chromatography
HS_RM	structured lipids produced from high-oleic sunflower oil under action of Lipozyme RM IM
HS_TL	structured lipids produced from high-oleic sunflower oil under action of Lipozyme TL IM
Hz	Hertz
i.d.	internal diameter
i.e.	id est
IM	immobilized
IPOo	chemical interesterified palm olein
IRRPW	irradiation pulse width
IUPAC	International Union of Pure and Applied Chemistry
IV	iodine value
KOH	potassium hydroxide
L	polyunsaturated fatty acid acyl chain (in NMR spectrum)
LB	line broadening
LDL-C	low-density-lipoprotein cholesterol
LLL	trilinoleoylglycerol
LSD	Fisher's Least Significant Difference test
L(x)	Lorentzian function
Mg	magnesium
M_o	equilibrium magnetisation
MUFA	monounsaturated fatty acid
m/v	mass/volume ratio
M(x)	hybrid function of Lorentzian and Gaussian functions
M_y	transverse magnetisation
M_z	longitudinal magnetisation
NA	not applicable
ND	not detected

NMR	nuclear magnetic resonance
NOE	Nuclear Overhauser enhancement
NRC	National Research Council
NUFA	number of unsaturated fatty acids
O	*cis*-9 monoene fatty acid acyl chain (in NMR spectrum)
OLL	1,2-dilinoleoyl-3-oleoylglycerol
OOL	1,2-dioleoyl-3-linoleoylglycerol
OOO	trioleoylglycerol
PD	pulse delay
pH	-log $[H^+]$
PMF	palm mid fraction
PN	partition number
POo	palm olein
POO	1,2-dioleoyl-3-palmitoylglycerol
POP	1,3-dipalmitoyl-2-oleoylglycerol
PO_RM	structured lipids produced from palm olein under action of Lipozyme RM IM
POSt	1-palmitoyl-2-oleoyl-3-stearoylglycerol
PO_TL	structured lipids produced from palm olein under action of Lipozyme TL IM
ppm	parts per million
PPP	tripalmitoylglycerol
PUFA	polyunsaturated fatty acid
PW	observation pulse width
qCNMR	Quantitative ^{13}C NMR
RBD	refined, bleached and deodorised
RM	*Rhizomucor miehei*
S	saturated fatty acid acyl chain (in NMR spectrum)
SAL	sal stearin
SD	standard deviation
Sdn.	Sendirian (private)
SEM	standard error of the mean
SFA	saturated fatty acid
SFC	solid fat content
SL	structured lipids

sn	stereospecific-numbering
S/N	signal-to-noise
SOY	soybean oil
SSS	saturated-saturated-saturated fatty acid profile
StOP	1-stearoyl-2-oleoyl-3-palmitoylglycerol
StOSt	1,3-distearoyl-2-oleoylglycerol
StStSt	tristearoylglycerol
SUS	saturated-unsaturated-saturated fatty acid profile
t	repetition time
T_o	Onset temperature
T_f	Offset temperature
T_1	spin-lattice relaxation time
T_2	spin-spin relaxation time
TL	*Thermomyces lanuginose*
UUU	unsaturated-unsaturated-unsaturated fatty acid profile
V	*cis*-11 monoene fatty acid acyl chain (in NMR spectrum)
viz.	videlicet
WHO	World Health Organisation