Regulation of Mammary Stearoyl-CoA Desaturase and the Effects on Milk Fat Composition in Lactating Mice

by

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Errata

Page xi,

'...Bicichoninic acid ...' should read '...Bicinhoninic acid...'

insert

sed is standard error of the mean.

Page 1, paragraph 2, line 5

'...much more...' should read '...made more...'

'...54.' should read '...24.'

Page 3, paragraph 3 line 1

'...random glycerol distribution...' should read '...random distribution...'

Page 13, paragraph 3, Line 1

'...clofibrates...' should read '...clofibrate's...'

Page 22, penultimate line

'...heirachy...' should read 'hierachy..'

Page 23 line 6

'....in the EPA... ' should read '...in that EPA...'

Page 27, line 6

'...prolifelators...' should read '...proliferators...'

Page 28 paragraph 2, line 8

'...half-life of SCD...' should read '... half-life of SCD mRNA...'

Page 29 paragraph 2, line2

'...discountered...' should read '...discounted..'

Page 33, line 5 and paragraph 3 line 7; page 52, line 3

'...by manufacturere...' should read '...by the manufacturer...'

Page 36 line 5

'...for at 480...' should read '...at 480...'

Page 37, paragraph , line 4

'...a further for...' should read '...a further...'

Page 37 last line; 43, last line; caption to Table 3.6; page 52 , line 10

'...data was...' should read '...data were...'

Caption Table 3.6, last line

'...was...' should read '...were...'

Line 6

'...agreement to...' should read '...agreement with...'

Page 68, Line 6

'...avtivity...' should read '...activity...'

Table 5.6, column heading

'Mammary... ' should read 'Liver...'

page 85, Beaulieu reference

'...effcets...' should read '...effects...'

page 86,penultimate line

'...fatyy...' should read '...fatty...'

Page 97, line 13

'...press...' Should read '...pressure...'

Abstract

The research described in this thesis tested, in the lactating mammary gland of female Swiss mice, a model for the control of lipogenesis developed for the liver of male mice. In male mice feeding a fat free diet, or a diet containing 0.5% w/w clofibrate, induces hepatic stearoyl CoA desaturase (SCD) mRNA transcription, which increases SCD activity and the amount of oleate incorporated into membrane phospholipids and the triacylglycerols of liver lipoprotein.

In a preliminary trial, SCD mRNA in liver and mammary gland and fatty acids (FA) in the liver, mammary gland and milk fat were measured in three groups (n=3) of lactating mice fed either a control diet or the control diet with added clofibrate (0.05% w/w) or a fat free diet. Concentrations of SCD mRNA in liver and mammary gland and proportions of individual FA in liver, mammary gland and milk were not significantly different between the control and clofibrate groups. There were, however, positive linear correlations between liver SCD mRNA and hepatic 16:1/16:0 FA ratio ($r = 0.495$, $P<0.05$), 18:1/18:0 FA ratio ($r = 0.520$, $P<0.05$) and milk 16:1/16:0 FA ratio ($r = 0.552$, $P<0.05$).

In a second trial, four groups (n=6) of lactating Swiss mice were used to compare the effect of clofibrate ingestion (control diet v. diet containing clofibrate (0.05% w/w)) and clofibrate injection (olive oil vehicle subcutaneously v. 15 mg clofibrate/100g LW in olive oil subcutaneously) for 7 days. Mammary SCD mRNA, but not liver SCD mRNA, was induced by ingested and injected clofibrate ($P<0.05$), compared to their control treatments. FA composition of liver, mammary gland and milk was not affected by either treatment. Correlations between mammary SCD mRNA and mammary tissue 16:1/16:0 FA ratio ($r = 0.660$, $P<0.05$), and 18:1/18:0 FA ratio ($r = 0.59$, $P<0.05$) were significant in the group ingesting clofibrate. Liver SCD mRNA for both treatments and mammary SCD mRNA for the injected group were not significantly correlated with FA composition. It was concluded that female mice that are lactating may be less sensitive to the effects of clofibrate than male mice.

In the preliminary trial, SCD mRNA transcription was induced ($P<0.05$) 2.1 fold in the mammary gland and 5.3 fold in the liver ($P<0.05$) of the mice fed the fat free diet over the control treatment. Induction of transcription was not transmitted to an effect on the FA composition of the liver, mammary gland or milk. However, there was a trend ($P<0.10$) for

milk 16:1/16:0 FA ratio to be increased in the fat free treatment over the control treatment. Liver SCD mRNA was correlated ($r = 0.552$, $P<0.05$) with milk 16:1/16:0 FA ratio, liver 18:1/18:0 FA ratio ($r = 0.520$, $P<0.05$) and liver 16:1/16:0 FA ratio ($r = 0.61$, $P<0.05$). In a third trial, lactating Swiss mice were allocated to three groups (6 mice/group) which were either fed a fat free diet, a safflower oil diet (25% w/w) or an olive oil diet (25% w/w) over a 7 day period. The safflower oil diet was included because polyunsaturated FA inhibit SCD activity in the liver while a fat free diet stimulates its activity. The olive oil treatment was included as a reference point with which to compare the responses to the other treatments. In the event, the intake of polyunsaturated FA by the mice on this diet may have been sufficient to inhibit the induction of SCD mRNA so that only relative responses between the various diets could be considered.

Mammary SCD and liver mRNA transcription levels were greater in the fat free treatment ($P<0.05$), compared with the olive and safflower oil treatments. Mammary SCD enzyme activity was not significantly affected by treatment., The fat free treatment increased liver 16:1/16:0 FA ratio and 18:1/18:0 FA ratio ($P=0.05$) and the mammary 16:1/16:0 FA ratio ($P<0.05$) but not the 18:1/18:0 FA ratio compared with the other two diets. The olive oil treatment increased palmitoleate and oleate concentration in the liver, mammary gland and milk ($P<0.05$). The increase in the concentration of oleate reflected the composition of the olive oil in the diet. Similarly, dietary intake influenced the significantly greater proportion of linoleate in the milk of the safflower treatment ($P<0.05$). The oleate concentration, 16:1/16:0 and 18:1/18:0 FA ratios were greater ($P<0.05$) in the milk of the group fed the fat free diet than those in the milk of the group on the safflower oil diet. An accumulation of stearate ($P<0.10$), indicating SCD inhibition, was present in the milk of the safflower oil treatment compared to the fat free treatment. The proportion of saturated fatty acids from octanoate to palmitate was greater in the milk from the mice on the fat free diet compared with those on the safflower oil treatment. The proportions of long chain fatty acids of molecular weight greater than linoleate were higher in the milk from the mice fed the diets containing the oils.

Acknowledgements

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Throughout the experience of gaining this qualification, I have met and been befriended by many different people. Most will be remembered for different reasons, but one person who stands out and deserves special mention, is Megan Callaghan. Megan, you have made integration into the lab and Ruakura social life easy. You listened to all my problems, mostly 'non-scientific', gave good advice and even chewed my ear out when it was needed. I hold our friendship in high regard.

Finally, I wish to thank my family. Thank you Mum and Dad for listening to my ceaseless drivel when it seemed all my existence was just about writing this thesis. To my brother, sister-in-law and little Chloe, you guys being over here for Christmas gave me the tonic I needed to finish.

Development of chronic disease depends on genetic responses to the environment for which the diet is a major influence (Paisley et al. 1996). Within the diet, the type of dietary fat has the potential to alter body weight and composition and also influence the onset and progression of various chronic diseases in human beings (Clarke and Jump 1996: Waters et al. 1997). Dietary fat affects membrane composition and fluidity as well as increasing cell metabolism and division rates (Ntambi 1995). The nutritional functionality of fats is influenced by its chain length, the degree of unsaturation, the type of isomer (cis/trans) and the position of the FA on the glycerol backbone (Kaylegian 1995). Saturated FA in the sn-1 and sn-3 positions of TAG can exhibit different metabolic patterns due to their low absorptivity. This means dietary fats with saturated FA in the sn-1 and sn-3 positions (cocoa butter and oil, palm oil) can have different biological consequences than those fats (milk fat) in which the saturated FAs are primarily in the sn-2 position (Decker 1996).

While there is a positive relationship between saturated FA intake and adverse lipoprotein cholesterol concentrations in humans (German et al. 1997; Vanden Havel 1997), this relationship is not seen with mono-unsaturated FA. Therefore, a reduction in palmitate, myristate and laurate while increasing mono-unsaturated FA (eg. oleate) would make milk products, like butter, a more nutritionally attractive product. Unlike other saturated FA, stearates lack of artherogenic properties has been attributed to its rapid conversion to oleate (Decker 1996).

Nutritional guidelines recommend the consumption of 30% or less of dietary energy as fat and less than 10% of dietary energy from saturated FA (Vanden Havel 1997), of which only 20% of New Zealand people meet these recommendations (Metcalf et al. 1998). For this reason butter, with high total and saturated fat content, has suffered strong criticism from the medical community (Jimenez-Flores 1997). Much of this criticism is not warranted. When butter provides 20-40% of dietary energy it is unquestionably hypercholesterolaemic. However, all products (not just butter) based on milk fat, provide only about 5% of dietary energy in a typical Western diet (Fumeron et al. 1991).

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List of Abbreviations

ACC	Acetyl-CoA carboxylase
ACO	Acetyl-CoA oxidase
ANOVA	Analysis of variance
bp	Base pairs
BCA	Bicichoninic acid
CLA	Conjugated linoleic acid
dATP	Deoxyadenine triphosphate
dGTP	Deoxyguanosine triphosphate
dTTP	Deoxythymidine triphosphate
DEPC	Diethyl pyrocarbonate
DHA	Docosahexanoic acid
DNA	Deoxyribonucleic acid
EDTA	Ethylenediaminetetra-acetic acid
EPA	Eicosapentanoic acid
FA	Fatty acid
FAS	Fatty acid synthase
FABP	Fatty acid binding protein
FAMES	Fatty acid methyl esters
GAPDH	Glyceraldehyde-3-phosphate dehydrogenase
LPL	Lipoprotein lipase
LT	Leukotrienes
ME	Malic enzyme
MF	Milk fat
MFGM	Milk fat globular membrane
mRNA	Messenger ribosomal nucleic acid
MOPS	3-[N-morpholino]propane-sulfonic acid
NaCl	Sodium chloride
NADH	Nicotinamide adenine dinucleotide
NADPH	Nicotinamide adenine dinucleotide phosphate
PG	Prostaglandins
PP	Peroxisomal proliferator

PPAR	Peroxisomal proliferator activator receptor
SCD	Stearoyl-CoA desaturase
SDS	Sodium dodecyl sulphate
SSC	Sodium chloride, sodium citrate buffer
TAG	Triacylglycerol
TCA	Tricarboxylic acid cycle
Thio II	Thioesterase II
UV	Ultra-violet