Review

Long-chain fatty acid metabolism in fasting and diabetes: relation between altered desaturase activity and fatty acid composition

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Metabolism of long-chain fatty acids is impaired in both fasting and diabetes mellitus. A decrease in insulin activity simultaneously reduces the activity of the delta-9, delta-6, and delta-5 desaturases with respect to fatty acids either synthesized by the animal (palmitic and stearic acids) or dietarily essential (linoleic and alpha-linolenic acids). Insulin therapy corrects this defect and directly influences the level of the desaturase proteins, not only controlling the synthesis of new desaturase enzyme but also stabilizing the existing enzyme by a mechanism that is not yet understood. Although in general changes in tissue fatty acid composition are usually thought to reflect such a condition of altered desaturase activity, in diabetes there is no simple relation between the changes in desaturase activity and the changes in fatty acid composition. Fatty acid composition depends not only on desaturation/elongation but also on other interacting aspects of lipid metabolism including oxidation, substrate availability, acyl exchange, and prostanoid synthesis, as well as dietary and hormonal status. The frequent nonagreement between desaturase data and fatty acid composition in diabetes suggests that these other factors play as important a role in determining the effects of diabetes on fatty acid composition as does impaired desaturation/elongation. Further work is necessary to understand and resolve the apparent discrepancies between altered activity of enzymes controlling metabolism of long-chain fatty acids and abnormal membrane phospholipid composition in diabetes.

Keywords: fatty acid; desaturation; elongation; diabetes; insulin; glucagon; fasting

Introduction

Long-chain fatty acids are vital components of mammalian cells, providing an important store of energy and also playing a leading role in membrane structure and function. Genetically induced diabetes in humans and animals or experimentally induced diabetes in animals is associated with a variety of metabolic derangements manifested by defects in the utilization of carbohydrate, in the synthesis and catabolism of protein, in the metabolism of lipids, and by acid-base disturbances. Administration of appropriate doses of insulin generally restores these metabolic functions to within normal limits. Research on lipid metabolism in diabetes has provided a wealth of information on changes in long-chain fatty acid composition and metabolism as well as the importance of insulin in these processes.

The fatty acid composition of membranes and whole organs is necessarily dependent on desaturation/elongation. Furthermore, factors that alter desaturation, e.g., fasting and diabetes, also affect fatty acid composition. However, it is not clear that one necessarily sees a similar change in enzyme activity and fatty acid composition simultaneously. This review addresses the relation between changes in desaturase activity and changes in fatty acid composition in fasting and diabetes as influenced by factors such as insulin and glucagon. It is our impression that although there are many consistencies between these two types

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of data, several studies also clearly indicate that changes in desaturase activity are only one factor governing membrane or tissue fatty acid composition.

Desaturation and elongation

While some long-chain fatty acids can be synthesized de novo in animals, others have to be obtained from plant sources. Palmitic (16:0), palmitoleic (16:1*n*-7), stearic (18:0), and oleic acids (18:1*n*-9) have a dual origin, both exogenous and endogenous. Linoleic (18:2*n*-6) and alpha-linolenic (18:3*n*-3) acids originate from the diet, e.g., they must be ingested and are then converted endogenously by the animal to longer-chain fatty acids with higher unsaturation that are structurally essential to the animal, e.g., arachidonic acid (20:4*n*-6) and docosahexaenoic acid (22:6*n*-3) (*Figure 1*). Synthesis of arachidonic acid and docosahexaenoic acid, which has been especially studied in rat liver, is controlled by an alternating sequence of desaturation and elongation steps. The main sequence of reactions in the series is the one beginning by a delta-6 desaturation and followed alternately by elongation, delta-5 desaturation, another elongation, and delta-4 desaturation. The same enzymes are generally considered to desaturate and elongate linoleic, alpha-linolenic, oleic, and palmitoleic acids, evoking the corresponding longer-chain fatty acids. Delta-9, delta-6, and delta-5 desaturase activities are highly dependent on nutritional and hormonal factors.[1,2] As a result of the close association between carbohydrate and lipid metabolism, it is not surprising that much of the hormonal study of these enzymes has been focused upon the action of glucagon and insulin in the liver. Although palmitoleic and oleic acids are not dietarily essential, delta-9 desaturation of stearic and palmitic acids is an important reaction in all tissues.

Delta-6 desaturation is the rate-limiting step in the biosynthesis of polyunsaturated fatty acids; the relative rates of the delta-6 and delta-5 desaturases are equal in normal rats.[2] Delta-4 desaturase has been studied very little, the conversion of adrenic acid to docosapentaenoic (22:5*n*-6) acid occurring in liver only to a limited extent. However, this conversion may be important in the adrenal and testis. The delta-4 desaturation of docosapentaenoic acid (22:5*n*-3) to docosahexaenoic acid (22:6*n*-3) is important in the central nervous system.[2] Essential fatty acids of both the *n*-6 and *n*-3 series have an indispensable structural function as integral parts of biomembrane phospholipids. In addition to their structural function, dihomo-gamma-linolenic (20:3*n*-6), arachidonic (20:4*n*-6), adrenic (22:4*n*-6), and eicosapentaenoic acids (20:5*n*-3) are also known precursors of prostanoids.

Fatty acid elongation proceeds by incorporation of malonyl-CoA into acyl-CoA chains. With unsaturated fatty acids, the rate of elongation is more rapid than desaturation, especially if the first double bond is between carbons 6 and 7 as in gamma-linolenic acid. Hence, there are usually low levels of elongase substrates in most organs. Linoleic, alpha-linolenic, and

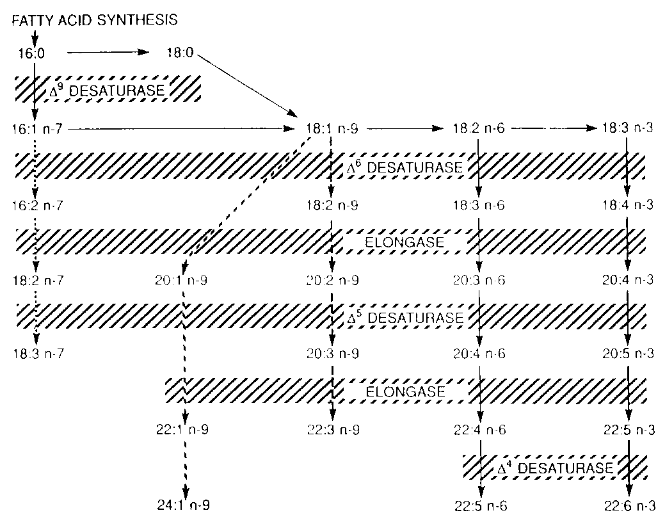

Figure 1 Pathway of metabolism of long-chain fatty acids in animals and humans (from ref. 51). The more unsaturated substrates are preferred at each desaturation step, so in the presence of sufficient amounts of dietary 18:2*n*-6 and/or 18:3*n*-3, longer chain metabolites of 18:1*n*-9 and 16:1*n*-7 are usually only found in very low amounts. Conversely, unless the desaturases are inhibited by nutritional conditions (fasting, zinc deficiency), hormones (glucagon, corticosteroids), or disease conditions (experimentally or genetically induced diabetes), dietary deficiency of 18:2*n*-6 and/or 18:3*n*-3 will result in the accumulation of longer chain metabolites of 18:1*n*-9, particularly 20:3*n*-9.

oleic acids, instead of being desaturated first by the delta-6 desaturase, may also first be elongated to the corresponding 20 carbon unsaturated acids. These acids may then be desaturated by a delta-5 desaturase, producing acids that, as far as is known, can only be further desaturated in rat testes (delta-8 desaturase).[1] The delta-9 desaturase is widely distributed in animals, including humans, and it is present in nearly all tissues. The delta-6 and delta-5 desaturases are more specifically localized. Their presence in mammals is not ubiquitous, and their activity varies broadly with the kind of tissue and species considered. They are functionally absent in the cat family.[1]

In addition to chain desaturation and elongation reactions involved in saturated and polyunsaturated fatty acid transformation in animals, retroconversion may play a role in the conversion of long-chain unsaturated acids to acids of shorter chain length. Oxidative degradation, produced by mitochondria and peroxisomes of ingested fatty acids, may also play an important role in the selection of tissue fatty acids. Since fatty acids are mostly esterified to lipid molecules, the existence, activity, and specificity of fatty acid esterification and hydrolyzing reactions also play a noticeable role in determining the final composition of tissues.

Fasting

Fasting and experimental diabetes involve an imbalance between caloric availability and requirement. Both appear to have similar effects on enzyme synthesis, liver glycogen, and several aspects of lipid metab-

Table 1 Effect of fasting/refeeding on delta-9, delta-6, and delta-5 desaturation of radiolabeled long-chain fatty acid substrates in rat liver microsomes

Substrate	Control	Fasted	Refed	Reference
Stearic acid	30.7	1.4	17.3	3
		15.8	35.6	4
	20.6	2.4–3.6		14
Linoleic acid	22.0	11.0		8
	7.8	3.4	5.8	3
		8.6	20.4	12
Alpha-linolenic acid		26.6	42.1	12
		20.1	36.4	7
Dihomo-gamma-linolenic acid[a]	14.1	13.4–26.2		13

Note: Data on each row is for a different study as referenced on the right. Results are expressed as % of labeled substrate desaturated. Fasting varied from 12–96 hours and the refeeding period was 12–48 hours.
[a] HTC cells in culture.

olism including desaturation and elongation. Particularly well known is the positive response of hepatic microsomal delta-9 desaturation activity to refeeding after fasting (*Table 1;* refs. 3 and 4). Studies with radiolabeled stearic acid have clearly shown not only an increase in the total radioactivity incorporated into the refed rats but also an increase in the amount of oleic acid radioactivity present at different times, suggesting increased desaturation may be coupled to decreased oxidation of long-chain fatty acids during recovery from fasting.[4]

Several studies have demonstrated that fasting decreases both delta-6 desaturation and elongation of linoleic acid (refs. 5–7; *Table 1*). Liver microsomal desaturating activity of stearic and linoleic acids is largely recovered by simply refeeding animals for 12 hours with 10% glucose in the drinking water after starvation for 48 hours. Refeeding also restores the activity of alpha-linolenic acid desaturation to normal values (*Table 1;* ref. 7). Fasting and refeeding evoke their deactivation-activation effects upon the microsomal desaturase enzyme system itself and not upon a cytosolic protein cofactor.[7] Both fasting and increased intake of carbohydrate decrease while a fat-free diet increases linoleic acid delta-6 desaturase activity.[3,5,8-11] In fasted rats that are refed a glucose diet, delta-6 desaturase activity is reactivated for a short time and then falls again.[8] This transient increase has been interpreted as the effect of insulin secretion followed by active glycolysis.[12]

Lee and Sprecher[13] have demonstrated that delta-5 desaturation was not significantly modified in rats maintained on a fat-free diet and then fasted for 24 hours; however, after 48 hours fasting, a 50% decrease in delta-5 desaturase activity was observed (*Table 1*). Poisson[6] reported a decrease in microsomal liver delta-5 desaturation in rats fed a fat-free diet and then fasted for 96 hours. De Alaniz et al.[14] have shown that in HTC cells, fasting evokes an increase in delta-5 desaturase activity that is quickly restored to normal when the cells were maintained in a calorically complete medium. Twelve hours of fasting produced a significant increase in delta-5 desaturase activity of liver microsomes of rats fed a balanced diet. This increase was maintained for 48 hours and then declined to control values after 72 hours of fasting (*Table 1*). Based on the different fasting periods, it was observed that linoleic, arachidonic, and docosahexaenoic acids increased in total lipids of fasted HTC cells as well as in microsomes of fasted rats.[14] This is not necessarily consistent with the increased delta-5 desaturase activity they reported, though De Alaniz et al.[14] suggested that the relative increase of linoleic acid could be responsible for the increased desaturation of dihomo-gamma-linolenic acid that led to the increased synthesis of arachidonic acid thus avoiding the accumulation of intermediate acids.

Although diabetes causes increased energy expenditure and increased appetite, reducing food availability to diabetic rats actually reduces the diabetes-induced changes in linoleic and arachidonic acid composition (see next section). Hence, it is clearly important to distinguish the roles of food intake and diabetes itself on changes in both desaturation and fatty acid composition.

Insulin, glucagon, and chemically induced diabetes

Impaired synthesis of long-chain fatty acids in the alloxan-diabetic rat was reported by Stetten and Boxer, who observed that incorporation of deuterium from labeled body water into liver and carcass fatty acids was depressed in diabetic animals as compared with normal rats.[15] Subsequent studies with radiolabeled water given to depancreatized rats in in vivo, as well as in vitro studies using 14-C-glucose or acetate, have clearly shown that fatty acid synthesis is severely depressed in alloxan-diabetes and that the defect is reversible by administration of insulin. Acetyl-CoA carboxylase activity is also severely inhibited in the

diabetic animal. Insulin administered in vivo or incubated with glucose and adipose tissue slices in vitro inhibits fatty acid release.[15] Insulin also corrects the decrease in de novo synthesis of palmitoleic and oleic acids observed in older rats compared with younger ones.[15]

Other studies have shown that although the amount (mole %) and synthesis of palmitoleic and oleic acids by epididymal fat was markedly depressed in alloxan-diabetes, the depressed synthesis was not corrected by insulin in vitro. Pretreatment of the diabetic animals with insulin restored synthesis of the unsaturated fatty acids completely.[15] The insulin-correctable block in oleic acid synthesis in the diabetic animals was at the level of the microsomal enzymatic conversion of stearate to oleate (ref. 16; *Table 2*). However, there was a delay in the appearance of the enzyme activity and only at 24 hours after the administration of insulin did it return within the normal range. Subsequently the capacity to convert stearic acid to oleic acid was maintained for 48 hours or longer and then abruptly declined.[17]

There is a relationship between the dosage of insulin and the quantity of enzyme activity induced as well as the duration of the insulin effect. The marked increase in desaturation induced by insulin appears to be due either to activation or to synthesis of new enzyme protein.[15] In order to restore liver microsomal stearoyl-CoA desaturase activity within 24 hours, streptozotocin diabetic rats require 2 iu of regular insulin every 6 hours. Diabetic animals treated with subcutaneous injection of 4 iu of NPH insulin twice a day show an increase in delta-9 desaturase activity above baseline at longer time intervals.[16]

As one of the countercurrent hormones, glucagon is antagonistic to insulin, which is clearly seen by its inhibition of delta-6 and delta-5 desaturation in rat liver microsomal preparations.[9,10,16,17] Glucagon also prevents the refeeding-induced activation of delta-6 desaturation in previously fasted rats. The mechanism of glucagon's inhibitory effect on desaturation is thought to occur through stimulation of adenyl cyclase and increased intracellular cyclic AMP because dibutyryl cyclic AMP induces the same effect as glucagon.[11] Comparable effects on fatty acid composition are not known.

The in vitro effects of glycerol upon the enzymatic capacity of isolated diabetic hepatocytes to convert stearic to oleic acid was studied by Mercuri and De Tomas.[4] Dietary glycerol was able to restore delta-9 desaturase activity depressed by the diabetic state. The presence of glycerol in the incubation medium of isolated rat hepatocytes stimulated not only fatty acid incorporation but also promoted stearic acid conversion to oleic acid. When comparing the effects of a low-fat fructose diet to a low-fat glucose diet, Worcester et al.[18] have shown that fructose increases stearoyl-CoA desaturase activity in the liver of both normal and streptozotocin-diabetic rats. Diabetes decreased this enzymatic activity in rats fed either diet.

The fatty acid composition of plasma and liver phospholipids and triglycerides of lean diabetic rats had a higher content of polyunsaturated fatty acids, especially linoleic acid. With the exception of plasma phospholipids, there was an increase in the stearic acid/oleic acid ratio in liver phospholipids and plasma and liver triacylglycerols, indicating impaired desaturation in diabetic rats. Thus, enzyme data for delta-9 desaturation[4,15-18] and stearate/oleate fatty acid data[18] are in agreement.

In addition to altered linoleic acid content, abundant evidence also exists for altered linoleic acid metabolism in experimental diabetes. Decreased incorporation of 1-14-C acetate into fatty acids has suggested an impairment of arachidonic acid synthesis in the epididymal fat pad of alloxan-diabetic rats, leading to an accumulation of linoleic acid. However, multiple metabolic pathways for acetate metabolism make it difficult to interpret these results.[19] Mercuri et al.[20] have extended this initial observation and have shown that the desaturation of linoleic acid to gamma-linolenic acid is significantly depressed in liver microsomes from alloxan-diabetic rats (*Table 2*). The defect in this instance appeared localized at the terminal desaturase protein and was corrected by pretreatment of the animals with insulin.[3,20] Friedmann et al.[21] have also shown decreased desaturation in liver and lung of alloxan-diabetic rats. However, arachidonic acid levels (%) in liver were unchanged in this study.[21] Furthermore, incorporation of radiolabeled linoleic acid into liver and lung (total counts) was decreased in the diabetic group. Thus, linoleic acid oxidation may be in-

Table 2 Effect of insulin and arachidonic acid on delta-9 and delta-6 desaturation of radiolabeled substrates by liver microsomes from alloxan-diabetic (A–D) rats

Substrate	A-D	A-D + Insulin	A-D + arachidonic acid[a]	Reference
Palmitic acid	25–70	96–125	43	3,20,24
Stearic acid	32–61	112–120	–	20,24
Linoleic acid	22–56	64–117	–	3,20
alpha-Linolenic acid	71	89	–	24

Note: Data for palmitic, stearic, and linoleic acids are given as a range because it summarizes results from several studies (cited on the right). Results expressed as % of control activity.
[a] Fat free diet with 100 mg/day oral ethyl arachidonate.

creased in diabetes, which may affect its availability for desaturation.

Eck et al.[16] have shown that at least 24 hours of insulin treatment, given in a dose sufficient to transiently lower blood glucose, is required to correct the defect in linoleic acid delta-6 desaturase activity of streptozotocin-diabetic rats, although this enzyme activity is diminished to a lesser extent than delta-9 desaturase activity. In cultured cells incubated in the presence of labeled alpha-linolenic acid during 24 hours, De Alaniz et al.[22] and Brenner[11] have shown that delta-6 desaturase activity was increased by insulin in a concentration-dependent manner, thus demonstrating that delta-6 desaturation is directly sensitive to insulin. However, insulin evoked different effects on the two routes of alpha-linolenic acid transformation: although it increased the delta-6 desaturation route leading to eicosatetraenoic acid[8,11,14,23] and eicosapentaenoic acid, it depressed the elongation pathway that forms eicosatrienoic acid (n-3) and eicosatetraenoic acid.[5,11,14,23] In alloxan diabetes, microsomal delta-6 desaturation of alpha-linolenic acid is also depressed but corrected by insulin treatment in only 2 days. Desaturation of palmitic and stearic acids was more responsive to insulin than was desaturation of alpha-linolenic acid (ref. 24; *Table 2*).

The 17 hours required to normalize desaturation after insulin was given to diabetic rats[16] is consistent with a reported peak in desaturase activity[25] and is attributable to an effect on synthesis of the terminal desaturase protein. The effect is apparently caused by enzyme induction, since inhibitors of protein synthesis impair the recovery of enzyme activity produced by insulin injection.[1-3,5,12,17,19,21] However, insulin does not increase delta-6 desaturase activity when injected into nondiabetic animals (refs. 25,26; *Table 3*); in fact, it promoted a small decrease in linoleate desaturation 3 hours after the injection.[27]

Poisson et al.[28] have demonstrated that with gamma-linolenic acid as the radioactive precursor, alloxan diabetes causes decreased synthesis of arachidonic acid in the liver, kidney, and whole rat, leading to the hypothesis that diabetes also causes a decrease in gamma-linolenic acid elongation in rats. In streptozotocin-diabetic rats that received the same tracer dose of either 14-C gamma-linolenic acid or 14-C dihomo-gamma-linolenic acid by stomach tube, the 14-C radioactivity incorporated into arachidonic acid was considerably decreased when compared to control rats.[26,29,30] Streptozotocin diabetes also partially inhibited the delta-5 desaturation of 14-C dihomo-gamma-linolenic acid in vitro, an effect that was reversed and actually overshot baseline activity with subcutaneous injection of moderate quantities of insulin.[26] These results[26] provide in vivo and in vitro evidence of insulin regulation of delta-5 desaturation as a secondary determinant of linoleic acid metabolism to arachidonic acid.

Diabetes has a greater effect on the delta-9 desaturase than on the delta-6 and delta-5 desaturases, thereby limiting the synthesis of oleic acid and therefore its derivative eicosatrienoic acid (20:3n-9). If dietary supplies of linoleic and alpha-linolenic acids are limited, e.g., by dietary deficiency, arachidonic and eicosapentaenoic acids cannot be adequately synthesized due to lack of substrate. However, oleic acid is synthesized in abundance and its longer-chain product, eicosatrienoic acid (n-9), is also synthesized from oleic acid. Since streptozotocin- or alloxan-induced diabetes impairs delta-9, delta-6, and delta-5 desaturation, diabetes actually lowers the eicosatrienoic acid/arachidonic acid ratio in phospholipids of the linoleic acid deficient rat liver despite decreasing the arachidonic acid/linoleic acid ratio (ref. 31; *Table 4*). Thus, as in zinc deficiency, which inhibits delta-6 and delta-5 desaturation but stimulates delta-9 desaturation, a low eicosatrienoic acid/arachidonic acid ratio of tissue phospholipids is not a reliable index of adequate linoleic acid intake or normal synthesis of arachidonic acid in diabetes.[32]

Mercuri et al.[24] have observed a decrease in arachidonic acid and an increase in linoleic acid content in the fatty acid composition of the total liver lipids of alloxan-diabetic rats after two weeks of alloxan injection, an effect consistent with impaired delta-6 and delta-5 desaturase activities. However, stearic and oleic acid content was not modified, which is inconsistent with the impaired delta-9 desaturase activity normally observed in alloxan diabetes (*Table 4*). Comparing the distribution of the fatty acids in liver

Table 3 In vitro delta-6 and delta-5 desaturation of radiolabeled substrates by liver homogenates of normal, diabetic, and insulin-treated (In) normal and diabetic rats fed fat-free diets

Condition	Insulin (iu/day)	Days post insulin	Substrate: Linoleic acid	Substrate: Dihomo-gamma-linolenic acid
Normal	–	–	100	100
Normal + In	4	7	99	89
Diabetic[a]	–	–	74	72
Diabetic + In	4	7	85	52
	1	2–3	96	131

Source: From ref. 21.
Note: Desaturation is expressed relative to the normal value (100%). Results are expressed as % of control.
[a] Streptozotocin-induced (40–60 mg/kg body weight).

Table 4 Ratios of stearic acid/oleic acid (18:0/18:1*n*-9) and linoleic acid/arachidonic acid (18:2*n*-6/20:4*n*-6) of liver lipids of control and streptozotocin- (S; 70 mg/kg) or alloxan-induced (A: 100 mg/kg) diabetic rats on different diets

Substrate		Diet	18:0/18:1*n*-9	18:2*n*-6/20:4*n*-6
Total Lipids	Control	Fat−	0.76	1.33
	A-Diabetic	Fat−	0.58–0.74	0.56–0.79
Phospholipids	Control	EFA−	0.73	1.24
	A-Diabetic	EFA−	1.08	0.88
	Control	COM	0.24	1.70
	A-Diabetic	COM	0.34	1.31
Microsomal Total Lipids	Control	COM	0.46	1.21
	S-Diabetic	COM	0.38	0.66[a]
	S-Diabetic + Insulin	COM	0.90[a]	0.90[a]
Phospholipids	Control	COM	0.26	2.16
	S-Diabetic	COM	0.31	1.16[a]
Phospholipids	Control	EFA−	–	1.19
	A-Diabetic	EFA−	–	2.27[a]

Source: Refs. 21, 24, 31, 33, 35.
Abbreviations: Fat−: fat-free diet; EFA−: EFA deficient diet; COM: commercial chow diet.
[a] $P < 0.05$ versus control.

phospholipids of normal and diabetic rats fed a normal or essential fatty acid–deficient diet, Friedman et al.[21] reported a similar fatty acid composition whatever the diet (*Table 4*). In both rat testes and epididymal fat, Brenner et al.[3] found a significant decrease in the percentage of palmitoleic acid, a significant increase in linoleic acid, and a decrease in the arachidonic acid/linoleic acid ratio in the tissues of diabetic rats compared with controls. There was no difference between diabetic animals receiving or not receiving exogenously injected arachidonic acid.

The major alterations in liver microsomal fatty acid composition found in streptozotocin-diabetic rats were decreased proportions of arachidonic acid and increased proportions of linoleic acid,[33] probably related to but necessarily resulting from impaired delta-6/delta-5 desaturase activity. The decrease in linoleic acid following insulin injection was consistent with the marked stimulation of delta-6 desaturation; however, the expected increase in arachidonic acid did not occur.[33,34] These changes were present in phosphatidylcholine and phosphatidylethanolamine, with very little change in fatty acid composition in the phosphatidylserine/inositol fraction.[34]

In streptozotocin-diabetic rats, the fatty acid composition of phospholipids of heart, liver, kidney, aorta, and serum is suggestive of an impairment of delta-5 desaturase activity but with, interestingly, no impairment in the desaturation of *n*-3 fatty acids.[35] In fact, there was an increase in the proportion of *n*-3 fatty acids indicating that although the proportion of some fatty acids must increase if others decrease, they may also be independent effects of diabetes on desaturation of the *n*-6 and *n*-3 series in these tissues.

In total phospholipids of liver, plasma, and heart of streptozotocin-diabetic rats the main fatty acid differences compared with controls are an increased proportion of linoleic and dihomo-gamma-linolenic acids and a decreased proportion of arachidonic acid. However, in several other tissues, neither arachidonic acid nor docosahexaenoic acid was significantly changed in the diabetic rats. An increase in the proportion of arachidonic acid and a reduction in the proportion of linoleic acid were observed in the skin of diabetic rats.[36]

These studies of alloxan- or streptozotocin-induced diabetes in the rat clearly suggest that insulin deficiency is associated with impaired delta-9, delta-6, and delta-5 desaturation. What is not as clear is the extent to which impaired desaturation is reflected by appropriate changes in fatty acid composition, e.g., increased stearic acid/oleic acid, linoleic acid/arachidonic acid, and alpha-linolenic acid/docosahexaenoic acid. Some fatty acid data support the desaturation data, and some do not. Therefore, it seems that different models, different degrees of insulin depletion, and different organs are affected differently in experimental diabetes. In addition, the degree of linoleic acid that may be required for oxidation in diabetes could also impact on its availability for desaturation and its depletion from tissues relative to arachidonic acid.[21] This is especially evident when the quantitative whole body losses of polyunsaturated fatty acids from the diabetic rat are calculated because these losses are mostly accounted for by lower linoleic acid and little quantitative change in arachidonic or docosahexaenoic acids.[36]

It is generally assumed that the fatty acid composition of tissue phospholipids, especially that of microsomal phospholipids, is determined by changes in desaturase activity occurring in such membranes. While we have no evidence to the contrary, the activity of

the desaturases may actually be determined by changes in the fatty acid composition of microsomal lipids rather than the reverse. The composition of the lipid-protein milieu in microsomal membranes probably governs desaturase activity, and, although there is little direct evidence for effects on desaturation of lipids other than cholesterol,[1] it is likely that mechanisms affecting fatty acid composition will also alter desaturation. In view of some of the inconsistencies we have reviewed here in streptozotocin- or alloxan-induced diabetes with respect to changes in fatty acid composition compared to changes in desaturase data, it may be worth bearing in mind that these diabetes-inducing agents may alter tissue fatty acid composition through effects not initially or directly related to desaturation, e.g., linoleate oxidation, acyl exchange, or phospholipid synthesis. Since diabetic rats have an increased energy requirement that is responded to in part by linoleic acid oxidation leading to whole body linoleic acid depletion,[21,36,37] this may directly affect microsomal lipid composition, thereby impairing desaturation. The time course of these effects on desaturation and microsomal lipid composition may be different, which might then account for apparent inconsistencies in fatty acid compositional and desaturase data when only one data point is collected.

Genetic diabetes

The Bio-Breeding (BB) genetically diabetic Wistar rat is the only reported example of spontaneous insulin-dependent diabetes in the rat and the only well-characterized spontaneous model in any species with destructive insulitis resembling the lesions described in human Type I (insulin-dependent) diabetes.[38] With a daily injection of insulin necessary for the survival of the BB-diabetic rat, the fatty acid composition of liver phospholipids and microsomal total liver lipids is not significantly different from controls, which is not in agreement with the previously reported data on chemical diabetes (refs. 29,33; *Table 5*). Mimouni et al.[39] have observed that stearic acid delta-9 desaturase activity is still inhibited 24 hours after an insulin injection to hypoglycemic female BB-diabetic rats.

Chanussot et al.[38] studied rat liver fatty acid composition and delta-6 and delta-5 desaturation in 21-week old female BB-diabetic rats (28 days after the onset of the disease) that were killed 20 hours after insulin injection while still hyperglycemic. Under these experimental conditions, liver microsomal delta-6 and delta-5 desaturase activities were unchanged in the BB-diabetic rats when compared with the controls. However, oleic and linoleic acid levels in hepatic microsomes of BB-diabetic rats were increased when compared with control rats, while stearic and arachidonic acids were decreased and docosahexaenoic acid remained unchanged. Thus, the altered liver fatty acid composition of the BB-diabetic rats was inconsistent with apparently normal desaturase activity in the same animals. The daily treatment with insulin may explain why delta-6 and delta-5 desaturase activity of BB-diabetic rats was not different from control rats but does not explain why the linoleic/arachidonic acid ratio was increased. The experimental conditions for insulin injections were sufficient to normalize desaturase activity in the BB-diabetic rats and required similar amounts of insulin and time for treatment as for chemical diabetes.[26] Insulin has been shown to stimulate fatty acid desaturation, probably via an increase in enzyme synthesis. This phenomenon may therefore be

Table 5 Ratio of stearic acid/oleic acid (18:0/18:1*n*-9) and linoleic acid/arachidonic acid (18:2*n*-6/20:4*n*-6) of liver lipids from female control and female BB-diabetic rats

Substrate		18:0/18:1*n*-9	18:2*n*-6/20:4*n*-6	Reference
Phospholipids	Control	0.13	2.44	39
	BB-diabetic—24 h[a]	0.21	1.83	
Microsomal	Control	0.39	1.76	
Total Lipids	BB-diabetic—24 h[a]	0.28	2.19	
Total Lipids	Control	0.33	1.29	38
	BB-diabetic—20 h[a]	0.85	0.59	
Microsomal	Control	0.28	1.39	
Total Lipids	BB-diabetic—20 h[a]	0.51	0.77	
Total Lipids	Control	0.47	1.58	38
	BB-diabetic— 3 h[a]	0.55	1.08	
	BB-diabetic—17 h[a]	0.69	1.39	
	BB-diabetic—48 h[a]	0.69	1.06	
Phospholipids	Control:	0.37	2.20	42
	BB-diabetic— 3 h[a]	0.39	1.21	
	BB-diabetic—17 h[a]	0.33	1.51	
	BB-diabetic—48 h[a]	0.32	1.47	

[a] Time after stopping last insulin injection.

related to effects on protein synthesis.[16] On the other hand, a decrease in the level of desaturation through insulin-deficiency–induced glycolysis has also been reported.[24,26]

It is interesting to note that Oshino and Sato[25] reported a peak of desaturase activity 17 hours after refeeding starved rats, which is consistent with the time required for restoration after insulin was given to diabetic rats and is also consistent with the appearance of an effect on protein synthesis. The activation of acyl-CoA desaturase by soluble proteins has been reported previously.[7,40] We are inclined to attribute these opposing effects of diabetes and insulin to their effects on such protein factors. In BB-diabetic rats, the amount of fatty acids in liver microsomal total lipids suggests an inhibition of delta-6 and delta-5 desaturation, but this is inconsistent with the measured desaturase activities, e.g., the decreased ratio of arachidonic to linoleic acid would have suggested a decreased synthesis of arachidonic acid. This phenomenon of nonagreement between unaltered desaturase activity but altered fatty acid composition could reflect an increased utilization of arachidonic acid not compensated for by increased synthesis because of insulin deficiency. Delta-6 and delta-5 desaturase activity could also be less depressed in spontaneous diabetes and less responsive to insulin than is delta-9 desaturase activity, as previously observed in streptozotocin-induced diabetes.[16]

Mimouni and Poisson (ref. 41 and unpublished data) have shown that delta-9, delta-6, and delta-5 desaturase activities were similarly defective in BB-diabetic rats during the hyper- and normoglycemic period and were restored during the hypoglycemic period that followed insulin injection. Delta-6 and delta-5 desaturase activities were significantly depressed (−64 and −76%, respectively) 48 hours after insulin injection (hyperglycemia), less depressed (−41 and −68%, respectively) 17 hours after insulin injection (normoglycemia), and similar to controls 3 hours after insulin injection (hypoglycemia); delta-9 desaturase, delta-6, and delta-5 desaturase activities were similarly inhibited (−50, −34 and −11%, respectively). Correction of the enzyme activities occurred rapidly and completely 3 hours after the insulin injection to BB-diabetic rats that had not received insulin during the previous 48 hours but was not paralleled by reduced glycaemia, e.g., the desaturase activities had normalized when the BB-diabetic rats were still hypoglycemic but decreased when they were normoglycemic. Inhibition of the desaturases increased from the normo- to the hyperglycemic period.[41]

Few significant differences from controls have been apparent in the fatty acid composition of liver total lipids, phospholipids, and microsomal total lipids of BB-diabetic rats (refs. 39,41,42; *Table 5*). During the hyper- and normoglycemic periods, but not during the hypoglycemic period, linoleic acid of BB-diabetic rat liver phospholipids increased while arachidonic acid decreased, which is consistent with previously reported results on streptozotocin- and alloxan-diabetes[35] and also consistent with defective delta-6 desaturation. Despite impaired delta-9 desaturation, the oleic acid/stearic acid ratio remained remarkedly unchanged in BB-diabetic rats compared with controls.[38]

Insulin treatment of 16-week old BB-diabetic rats was sufficient to maintain a relatively normal fatty acid composition of liver microsomal total lipids. However, this was not consistent with the altered desaturase activities at the different periods of glycemia. The decreased arachidonic acid/linoleic acid ratio after insulin treatment suggested decreased synthesis of arachidonic acid compensated for by an increased synthesis following the insulin-deficient state; this was consistent with impaired delta-6 desaturase activity. This was not the case with the oleic acid/stearic acid ratio, which was increased in the hyperglycemic and normoglycemic groups, suggesting increased synthesis of oleic acid when delta-9 desaturase activity was actually decreased.

Despite being insulin-deficient, the BB-diabetic rat appears to have abnormalities in in vitro fatty acid metabolism that are inconsistent with those of alloxan- or streptozotocin-induced diabetes, e.g., impaired delta-9 desaturation with little or no effect on delta-6 or delta-5 desaturation. In addition, the changes in fatty acid composition are not consistent between these two models nor are they consistent with the differences in enzyme activity. As discussed with respect to alloxan- or streptozotocin-induced diabetes, the poor agreement between the effect of a change in glycemia (induced by a different time period from the last insulin injection) on microsomal or whole liver lipids and desaturase activities implies that they are not closely linked in a direct cause-effect relationship. Rather, factors that alter membrane fatty acid composition independently of desaturation, e.g., acyl exchange and beta-oxidation, may have a pivotal role in the altered lipid biochemistry observed in BB-diabetic rats.

Diabetes in humans

In humans, diabetes mellitus is characterized by the level of dependence on exogenous insulin, e.g., insulin-dependent or insulin-independent. Studies of fatty acid composition in both types of diabetes have yielded inconsistent data; some suggest linoleic acid/arachidonic acid is increased in plasma lipids,[43-46] but others have found no such effect.[47,48] However, as in the animal models, data on fatty acid desaturation are not consistent with the fatty acid composition data. Metabolism of dihomo-gamma-linolenic acid in non-insulin-dependent diabetics is decreased relative to controls.[47] The desaturation of deuterium-labeled eicosa-5,11,14 trienoic acid to arachidonic acid has been measured in plasma lipids of newly diagnosed diabetics who had never received insulin. After insulin treatment, it was found that the concentration of deuterated arachidonic acid rose significantly from pretreatment values, indirectly suggesting that this hormone is involved in the regulation of delta-5 desaturation in humans.[44,45]

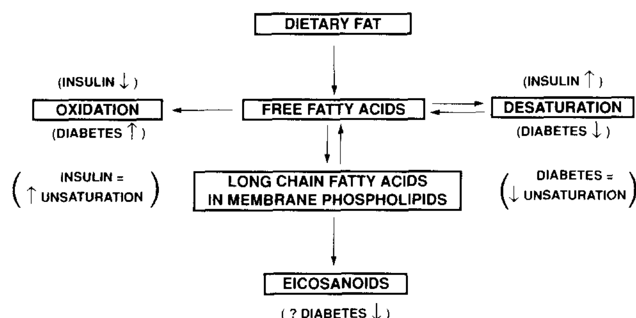

Figure 2 Schematic representation of the most widely studied abnormalities in long-chain fatty acid metabolism in diabetics. These changes have been observed in both animal models and in humans but are not necessarily consistent from one study to the next.

Low levels of prostaglandin E1 in platelets have been reported in diabetes.[48] Therefore, diabetics appear to have an abnormality of essential fatty acid and prostanoid metabolism that may lead to an increased requirement for the fatty acids that are dietarily essential, e.g., linoleic and alpha-linolenic acids. However, these fatty acids may not be metabolized normally in diabetes, e.g., increased linoleic acid oxidation may occur as observed in streptozotocin-induced diabetes.[21,36,37] These interactions in long-chain fatty acid metabolism in diabetics are shown in *Figure 2*. Attempts to overcome impaired desaturation by giving very large amounts of dietary linoleic acid, or by bypassing the blocked delta-6 desaturase by giving longer-chain metabolites such as gamma-linolenic acid and eicosapentaenoic acid have both provided clinically positive results.[43,49,50] Therefore, as in the animal models, data from human diabetes suggest that desaturation of linoleic acid and dihomo-gamma-linolenic acid is quite sensitive to insulin status without fatty acid composition necessarily being affected. Studies with close control of energy metabolism and assessment of long-chain fatty acid oxidation in human diabetes should provide the link between these apparently inconsistent data.

Conclusions

Substantial evidence from both animals and humans indicates that long-chain fatty acid metabolism is abnormal in diabetes and leads to impaired formation of labeled fatty acids dependent on delta-9, delta-6, and delta-5 desaturation as well as on chain elongation. Since the control of membrane lipid composition is multifactorial, other factors than changes in desaturation-elongation, e.g., membrane lipid degradation and synthesis, fatty acid oxidation, prostaglandin synthesis, and altered hormonal satus, may all be important in determining both fatty acid composition and desaturase/elongase activity (*Figure 2*). The influence of age and sex of the animals, duration of disease, diets,and/or fatty acids associated with insulin treatment, as well as type and duration of insulin administration, may also influence fatty acid metabolism during diabetes and insulin therapy. Further work is necessary to understand the insulin-dependent control of the synthesis of new desaturase protein and insulin's effect on the stability of the desaturase-lipid complex in membranes. The direct measurement of actual enzymes levels by specific antibodies raised against the enzyme protein should also offer promising results. Such studies are necessary to elucidate the mechanisms controlling microsomal desaturation and its dependence versus effect on the fatty acid composition of the membranes surrounding the desaturase enzyme complex.

Abbreviations

16:0 palmitic acid
16:1*n*-7 palmitoleic acid
18:0 stearic acid
18:1*n*-9 oleic acid
18:2*n*-6 linoleic acid
18:3*n*-3 alpha-linolenic acid
18:3*n*-6 gamma-linolenic acid
20:3*n*-9 eicosatrienoic acid
20:3*n*-6 dihomo-gamma-linolenic acid
20:4*n*-6 arachidonic acid
22:4*n*-6 adrenic acid
22:5*n*-6 docosapentaenoic acid
20:5*n*-3 eicosapentaenoic acid
22:5*n*-3 docosapentaenoic acid
22:6*n*-3 docosahexaenoic acid
BB Bio-Breeding diabetic rats

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