Studies of Incorporation of Radioactivity into Lipids by Human Blood

IV. Abnormal Incorporation of Acetate 1-C-14 into Fatty Acids by Whole Blood and Platelets from Insulin Independent Diabetics

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SUMMARY

The incorporation of acetate 1-C-14 into fatty acids by whole blood and platelets from insulin independent diabetics has been determined.

Whole blood from insulin independent diabetics incorporates significantly less C-14 into fatty acids than does whole blood from controls. As in previously reported studies done with blood cells from ketotic and nonketotic insulin dependent diabetics, a decreased percentage of fatty acid C-14 in palmitic acid (16:0) is found. This, however, exists as an isolated abnormality in whole blood from insulin independent diabetics. This abnormality may persist in some patients even when the fasting blood sugar is normal.

The incorporation of acetate C-14 into fatty acids by platelets from insulin independent diabetics is quite similar to that of the control group. However, the pattern of incorporation of C-14 into fatty acids is abnormal. Percentage of C-14 in both myristic and palmitic acids is decreased, while percentage of C-14 in saturated and unsaturated fatty acids with retention time corresponding to 20:0 or greater is increased. These abnormalities bear no relationship to the fasting blood sugar at the time of study.

These findings apparently mean that de novo synthesis of fatty acids is impaired in blood cells from both insulin dependent and insulin independent diabetics, while chain lengthening is relatively little affected. DIABETES *14*:709-15, November 1965.

We have shown in previous communications that whole blood and platelets from insulin dependent diabetics could be used to demonstrate abnormalities in fatty acid formation in uncontrolled[1,2] and even in quite well controlled insulin dependent diabetics.[3] By using this system, we were able to show the existence of qualitative abnormalities involving formation of 14 carbon and particularly 16 carbon fatty acids in whole blood and in platelets. The relative decrease in incorporation of acetate C-14 into these fatty acids was associated with a relative increase in incorporation of C-14 into longer chain fatty acids. Though the abnormalities were most marked in cells obtained from comatose diabetics, they could still be detected in platelets from insulin dependent diabetics, even when the fasting blood sugar was normal.[3]

These studies were all undertaken to test a basic hypothesis that diabetes, as it exists in man, is at least partially due to a genetic defect in ability to form certain fatty acids. If this hypothesis is reasonable, then it seems logical to look for this defect in any human cell, including blood cells which form fatty acids.

This hypothesis seems most susceptible to test in the insulin independent, rather than the insulin dependent, diabetic since levels of insulin or insulin-like activity have been found to be normal or increased[14,21,22] in these subjects.

Thus, if abnormalities in formation of fatty acids could be detected in isolated cells from these subjects, there could be only two general explanations: either the insulin in the plasma is for some reason ineffective, or the cells themselves are defective in their ability to form fatty acids.

Though we cannot determine as yet which of these rather gross alternative explanations is correct, it appears that abnormalities in formation of fatty acids can be detected in blood cells obtained from insulin independent diabetics.

METHOD AND MATERIALS

Five milliliters of heparinized whole blood or five milliliters of platelet-rich plasma prepared by centrifu-

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gation of heparinized whole blood at 450 G. were incubated with 5 μc of 1-C-14 acetate. All glassware was siliconized. Platelet counts were done by the method of Brecher and Cronkite.[4] If platelet suspensions contained more than 200 leukocytes per cubic millimeter, they were recentrifuged for two or three minutes. In this way, platelet suspensions containing 0-200 leukocytes per cubic millimeter were obtained. After four hours' incubation at 37° C., under 95 per cent oxygen and 5 per cent CO_2, lipids were extracted from whole blood or the platelets in plasma by the method of Folch et al.,[5] except that the crude lipid extracts were washed with an equal volume of 0.73 per cent saline solution to remove water soluble radioactive material. The lipids were saponified and extracted according to the method of Bjorntorp.[6] The fatty acids were methylated, using the method of Metcalfe and Schmitz.[7] The methyl esters were separated by gas liquid chromatography, using a twenty-foot, 20 per cent diethylene glycol succinate column. The fatty acid methyl esters in each peak and any material between the recognizable peaks were trapped at the column exit port in siliconized cellulose traps by a method developed in this laboratory.[8] This method yields 95-100 per cent recovery of known radioactive fatty acids. Radioactivity of each fatty acid was determined in a liquid scintillation spectrometer. Radioactive peaks were tentatively identified by their retention time relative to palmitic acid and by the use of known standard fatty acids. In some experiments, retention times were compared on a 20 per cent diethylene glycol succinate column at 215° C. and on a 3 per cent S.E. thirty column at 230° C.

All patients classified as insulin independent diabetics had never been in diabetic acidosis, nor had they required insulin to prevent ketosis. All had been hyperglycemic (FBS greater than 120 mg. per 100 ml. at some time). Only two had received oral hypoglycemic agents. The remainder were untreated.

RESULTS

The fatty acids which contain approximately 95 per cent of the total fatty acid radioactivity are listed in the tables. There are traces of radioactivity in various other odd and even chain saturated and unsaturated fatty acids, but the C-14 in these fatty acids rarely accounts for more than 5 per cent of the total.

Degradation studies of platelet fatty acids have shown that myristic and palmitic acids (14:0 and 16:0) are normally made by de novo synthesis, while fatty acids containing eighteen or more carbons are made by chain lengthening of a pre-formed fatty acid which contains relatively little radioactivity.[9]

Newly synthesized myristic and palmitic acids are found primarily in nonesterified fatty acids, while the radioactive fatty acids containing eighteen or more carbons are found almost solely in phospholipids and glycerides of platelets.[10]

Whole blood from insulin independent diabetics incorporated somewhat less C-14 into fatty acids than did whole blood from controls. However, differences are not significant (table 1). Nor are significant differences in C-14 in individual fatty acids found. However, there is a significant decrease in C-14 in the sum of radioactivity in 14:0 plus 16:0 in whole blood. There is also a significant decrease in radioactivity in short chain fatty acids (< 10:0 + 10:0). Since leukocytes are responsible for approximately two thirds of the incorporation of acetate C-14 into fatty acids by whole

TABLE 1 (*Continued on page 711*)

Total incorporation of C-14 into fatty acids by whole blood

	DPM incorporated per 5 ml. whole blood									
	Total incorporation	<10:0 + 10:0	12:0	14:0	16:0	14:0 + 16:0	18:0	18:1	18:2	20:0* + >20:0
Controls (16)										
Mean	117,364	491	2,630	14,892	26,053	40,956	15,258	7,910	946	45,670
S.D.	±47,592	±150	±1,149	±7,420	±12,613	±17,373	±10,479	±4,171	±597	±17,729
Insulin independent diabetics (11)										
Mean	87,646	251	1,964	10,828	15,763	26,588	12,909	6,327	790	35,631
S.D.	±31,870	±186	±887	±4,316	±5,695	±9,843	±6,429	±3,246	±356	±12,957
p value	.05-.10	.001-.005	.10-.20	NS	<.10->.05	.025	NS	NS	NS	.10-.20

*Fatty acids with retention time corresponding to arachidic acid (20:0) or greater.
NS = Not significant
S.D. = Standard deviation

blood,[11,12] data have also been compared on the basis of disintegrations per minute of C-14 incorporated into fatty acids of whole blood per 10^6 leukocytes. When compared on this basis, whole blood from insulin independent diabetics incorporates significantly less C-14 into fatty acids than does whole blood from controls. The decrease in incorporation of C-14 is not uniform, however. Radioactivity is significantly decreased only in 14:0, 16:0, and in short-chain fatty acids (table 1).

The exact meaning of a decrease in total fatty acid C-14 is difficult to determine. A decrease in total counts could be due simply to isotope dilution. It seems reasonable, however, that this should affect incorporation of C-14 into all fatty acids equally. Because the pool sizes of intermediates are unknown, these data should not be considered in terms of an increase or decrease in "synthesis" of a particular fatty acid. To eliminate as much as possible the effects of isotope dilution, data are also presented as percentage of total radioactivity in a particular fatty acid or group of fatty acids. When considered in this way, the pattern of incorporation of acetate C-14 by whole blood from insulin independent diabetics differs from normal only in having a decreased percentage of fatty acid C-14 in palmitic acid (16:0). No other significant differences are seen. There does not appear to be any relationship between the percentage of fatty acid C-14 in 16:0 and the fasting blood sugar at the time of study (table 2).

Platelets are surprisingly active in incorporation of acetate C-14 into fatty acids. If the platelet contribution to formation of fatty acids is subtracted from the total incorporation of acetate C-14 per 5 ml. of whole blood, a reasonable approximation of platelet as compared to leukocyte incorporation of C-14 into fatty acids can be made. This involves two assumptions: (1) isolated platelets are as active in formation of radioactive fatty acids as they are in whole blood, (2) the contribution of erythrocytes to formation of radioactive fatty acids in whole blood can be neglected. It can be calculated after making these assumptions that platelets have 1 to 2 per cent of the activity of leukocytes per cell in formation of radioactive lipids. Since not all platelet preparations were completely free from leukocytes, we have estimated the contribution which these leukocytes could make to the radioactivity recovered in fatty acids in experiments with platelet suspensions.

When the platelet contribution is subtracted from total radioactivity in fatty acids of whole blood, leukocytes are found to contribute a mean of approximately 1,300 dpm/10^6 leukocytes in insulin independent diabetics and 2,300 dpm/10^6 leukocytes in controls. The average contamination is 100 leukocytes or less per cubic millimeter, or approximately 500,000 leukocytes per 5 ml. of platelets in plasma. This number of leukocytes could be expected to contribute 2.3 to 3.6 per cent of the total fatty acid radioactivity of the platelet suspension—certainly a negligible contribution.

The total incorporation of acetate C-14 into fatty acids by platelets from insulin independent diabetics and controls is almost exactly the same (table 3). C-14 in 14:0 plus 16:0 is less in diabetics than in controls, and there is a trend toward more C-14 in long chain fatty acids. However, no significant differences are seen.

When the data are analyzed in terms of percentage of total fatty acid radioactivity in a particular fatty acid, or group of fatty acids, definite differences in pattern of incorporation are found, however (table 4). Platelets

TABLE 1 (*Continued from page 710*)

Total incorporation of C-14 into fatty acids by whole blood

	DPM incorporated per 10^6 leukocytes									
	Total incorporation	<10:0 + 10:0	12:0	14:0	16:0	14:0 + 16:0	18:0	18:1	18:2	20:0* + >20:0
Controls (16)										
Mean	3,486	15	83	435	790	1,225	430	223	29	1,375
S.D.	±1,490	±6	±41	±191	±439	±557	±241	±114	±21	±633
Insulin independent diabetics (11)										
Mean	2,311	6	49	284	440	703	338	159	21	953
S.D.	±1,145	±5	±20	±143	±266	±367	±202	±92	±14	±502
p value	.025-.05	<.001	.10-.20	<.05 >.025	<.05 >.025	.01-.02	NS	NS	NS	.05-.10

*Fatty acids with retention time corresponding to arachidic acid (20:0) or greater.
NS = Not significant
S.D. = Standard deviation

TABLE 2

Percentage of recovered CPM in various fatty acids of whole blood

Case	Subjects Race and sex	Age	Fasting blood sugar mg. per 100 ml.	Leukocytes/ cmm.	<10:0	10:0	12:0	14:0	16:0	16:1	14:0 + 16:0	18:0	18:1	18:2	20:0* + >20:0
Controls:															
FF	NM	41	71	15,800	ND	ND	ND	16.20	19.20	0.80	35.40	20.70	8.00	0.50	30.70
LW	NM	14	74	7,000	0.05	0.28	2.15	11.96	24.14	0.71	36.10	11.28	5.97	0.80	40.83
HH	NM	31	79	6,000	0.02	0.37	2.33	12.97	20.90	0.80	33.87	10.21	6.16	0.98	43.24
JR	NM	41	78	6,200	0.03	0.33	3.05	13.34	17.62	0.58	30.96	9.49	6.41	0.75	46.80
RM	WM	31	70	6,000	ND	ND	ND	5.40	35.00	2.20	40.40	20.00	8.00	1.60	24.90
CD	WM	63	84	7,700	0.06	0.36	2.77	14.44	18.77	0.45	33.21	12.56	9.84	0.55	38.36
KH	WM	26	67	5,200	0.03	0.40	2.32	13.49	19.88	1.00	33.37	10.71	4.58	0.56	44.15
AH	WM	37	93	4,800	0.06	0.62	1.82	14.62	25.89	0.65	40.51	11.14	5.15	0.68	37.09
JJu	WM	20	76	7,000	0.01	0.32	1.80	11.18	19.71	0.48	30.89	12.97	7.37	0.69	42.94
EL	WM	25	73	4,730	0.02	0.46	2.65	15.18	28.43	0.57	43.61	9.32	4.42	0.64	35.98
KA	OM	32	82	7,800	0.03	0.48	2.14	11.35	23.89	0.81	35.24	14.75	8.00	1.19	35.27
WC	OM	24	87	7,150	0.27	0.81	3.26	12.79	19.46	0.44	32.25	10.27	4.88	0.52	45.54
JT	NF	52	72	9,000	0.19	0.39	3.24	14.43	16.71	0.63	31.14	12.76	4.50	0.99	43.12
TS	NF	24	76	5,500	0.01	0.38	2.73	13.09	21.61	0.72	34.70	11.46	8.44	0.82	38.96
MA	NF	39	70	6,600	0.03	0.17	2.49	11.48	16.79	0.60	28.27	9.59	5.52	0.76	50.48
JJa	WF	22	76	8,340	0.47	0.56	1.56	10.52	27.99	1.17	38.51	9.80	8.44	0.75	34.58
Mean					0.09	0.42	2.45	12.65	22.25	0.79	34.90	12.31	6.61	0.80	39.56
Standard deviation					±0.13	±0.15	±0.51	±2.42	±3.77	±0.43	±4.01	±3.36	±1.60	±0.27	±6.25
Insulin Independent Diabetics															
JL	NM	35	93	9,400	0.03	0.08	0.93	29.60	20.60	0.78	30.21	14.66	6.11	1.03	44.04
RO	NM	44	113	12,000	0.05	0.45	3.99	15.73	21.09	0.87	36.82	10.41	9.49	0.72	35.24
CS	NM	50	79	4,600	0.02	0.08	1.34	11.03	15.61	0.53	26.64	17.01	8.17	1.06	43.24
AI	WM	35	123	8,800	0.20	0.83	3.30	12.88	17.93	0.80	30.81	9.88	13.96	0.80	36.91
NT	OM	35	89	6,300	0.14	0.33	2.09	10.67	14.95	0.67	25.62	8.16	6.78	1.40	50.60
BB	NF	68	111	7,400	0.02	0.04	2.14	10.69	19.12	0.45	29.81	17.14	5.58	0.94	42.08
MG	NF	40	203	5,100	0.06	0.10	2.08	14.81	26.58	0.51	41.39	12.40	2.47	0.67	37.40
CJ	NF	45	247	10,000	0.06	0.12	2.75	15.24	19.69	0.62	34.93	18.18	6.51	1.10	32.13
IL	NF	55	188	7,600	0.05	0.11	1.80	12.17	15.11	0.52	27.28	15.70	4.31	0.80	46.30
CR	NF	47	186	8,900	0.10	0.39	2.56	11.50	16.73	0.70	28.23	16.34	7.51	0.78	39.02
RJ	WF	47	121	12,100	0.03	0.15	2.20	11.36	14.87	0.49	26.23	15.71	10.04	0.64	41.76
Mean					0.07	0.24	2.29	12.34	18.39	0.63	30.72	14.14	7.36	0.90	40.79
Standard deviation					±0.05	±0.23	±0.81	±1.91	±3.40	±0.12	±4.77	±3.22	±2.94	±0.21	±5.06
p value					NS	.025-.05	NS	NS	.01-.02	NS	.025-.05	NS	NS	NS	NS

*Fatty acids with retention time corresponding to arachidic acid (20:0) or greater.
NS = Not significant
ND = Not detected separately

from insulin independent diabetics incorporate a significantly smaller percentage of fatty acid C-14 into 14:0 and 16:0. Differences in percentage of C-14 in 16:0 are particularly striking ($p < .01$). There is also an increase in percentage of fatty acid C-14 which is just barely significant ($p = .05$) in fatty acids with retention time corresponding to 20:0 or greater. It is interesting that the platelets from insulin independent diabetics incorporate a significantly greater percentage of fatty acid C-14 into an octadecadienoic acid (18:2). This was previously demonstrated in studies with whole blood from comatose diabetics.[1] It seems unlikely that the radioactivity is actually in linoleic acid. It is more likely that the radioactivity is in an octadecadienoic acid derived from oleic acid. Holloway et al.[13] have recently shown that this can occur. However, degradation of this fatty acid at the double bonds would be required to determine its structure.

DISCUSSION

Unlike the previously reported experiments with whole blood from insulin dependent diabetics, whole blood from insulin independent diabetics shows only an isolated decrease in percentage of C-14 in palmitic acid. It is interesting, but difficult to understand, why C-14 in myristic acid, which appears to have a similar mechanism of formation, does not also decrease. How-

TABLE 3

Incorporation of C-14 from 1-C-14 acetate into fatty acids by platelets

		Total Radioactivity Incorporated into Fatty Acids by 10^9 Platelets								
	DPM/10^9 Platelets	<10:0 + 10:0	12:0	14:0	16:0	14:0 +16:0	18:0	18:1	18:2	20:0+* >20:0
Controls (13)										
Mean	33,347	592 (152)†	1,004	6,303	8,748	15,051	2,291	881 (374)‡	210	12,242
S.D.	±12,059	±1529 (117)†	±488	±2,353	±3,025	±5,320	±1,085	±1,003 (±374)‡	±66	±4,561
Insulin Independent Diabetics (10)										
Mean	31,064	187	809	4,994	6,605	11,600	2,554	843	295	13,262
S.D.	±11,070	±229	±435	±2,714	±3,845	±6,491	±1,235	±813	±234	±5,013
p Value	NS	NS	NS	NS	<.20->.10	.10-.20	NS	NS	NS	NS

*Fatty acids with retention time corresponding to arachidic acid (20:0) or greater.
†See footnote on table 4.
‡See footnote on table 4.
NS = Not significant
S.D. = Standard deviation

ever, platelets from insulin independent diabetics do show a decrease in percentage of C-14 in myristic acid, although it is not as great as the decrease in percentage of C-14 seen in palmitic acid. In addition, platelets from these diabetics show a barely significant increase in percentage of C-14 in fatty acids containing twenty or more carbons. These qualitative changes can be detected even though total incorporation of C-14 into fatty acids by platelets from insulin independent diabetics and controls is similar.

The central and most constant abnormality in formation of fatty acids by blood cells from both insulin dependent and insulin independent diabetics thus appears to be a defect in formation of fatty acids by de novo synthesis. This defect in insulin independent diabetics does not appear to be related to the level of the fasting blood sugar at the time of study.

We can not at present determine the cause of these abnormalities. It is worth noting that a decrease in activity of the malonyl CoA pathway, particularly due to a decrease in acetyl CoA carboxylase activity, has been found in the liver of the untreated alloxan-diabetic rat.[15] In addition, the incorporation of malonyl CoA into fatty acids is depressed in the liver of the alloxan-diabetic rat.[16] However, the relevance of these findings which might be related to secondary factors, such as ketosis, acidosis or hyperlipidemia, to the findings of the present study is unclear. But, the possibility that the activity of the malonyl CoA pathway is less than optimal even when the fasting blood sugar is normal is an intriguing one and should be investigated.

Decreased TPNH has been implicated in the genesis of the defect in fatty acid synthesis in the liver of the alloxan-diabetic rat,[17] although Matthes et al. have found the level to be normal.[18] However, it is conceivable that TPNH could be utilized excessively in cells from diabetics for reduction of intracellular acetoacetate,[19] thus decreasing fatty acid formation even though the level of TPNH in the cell is normal. Though none of these diabetics was ketotic, the possibility that the intracellular level of acetoacetate might be increased should be considered, even though this possibility is extremely difficult to test. Acetoacetate added in vitro will, in fact, produce changes similar in many respects to those seen in cells from diabetics.[20]

Finally, the state of insulin in the blood of these patients must be considered. These cells do not appear to increase formation of fatty acids in response to insulin in vitro.[1] However, as was shown in previous papers, pattern of whole blood lipid formation returns to normal and pattern of platelet lipid formation is partially repaired after administration of insulin in vivo.[1-3] This may mean that insulin must exist in a form different from that in which it is added in vitro or perhaps the apparent response to insulin is actually due to inhibition of nonesterified fatty acid release or ketone formation or some other secondary effect.

The fasting level of insulin in plasma of diabetics of this type is reported to be normal or increased.[14,21,22] However, the physiologic effect of this insulin-like activity on fat formation is generally measured by oxidation of glucose 1-C-14 to C-14-O_2. This, though admittedly

TABLE 4

Percentage of recovered CPM incorporated into various fatty acids by platelets

Subjects				Platelets											
Case	Race and Sex	Age	Fasting blood sugar (mg. per 100 ml.)	(10^3/cmm.)	<10:0	10:0	12:0	14:0	16:0	16:1	14:0 + 16:0	18:0	18:1	18:2	20:0* + >20:0
Controls:															
FF	NM	41	71	228.7	0	0.56	3.47	15.79	25.65	0.29	41.44	10.29	1.15	0.54	38.59
RM	WM	31	70	247.5	0.05	0.15	2.56	18.71	27.58	0.64	46.29	7.52	1.58	0.85	37.70
JJu	WM	20	76	243.0	0	0.23	2.42	17.33	24.31	0.30	41.64	7.43	1.05	0.75	43.62
EL	WM	25	73	395.0	0.02	0.38	3.14	21.23	30.28	0.39	51.51	4.48	0.64	0.49	36.94
KH	WM	25	67	665.0	0	0.13	2.44	17.54	21.75	0.49	39.29	8.35	1.00	0.65	45.23
JR	WM	33	78	607.0	0.11	0.13	2.01	19.34	26.74	1.08	46.08	7.12	1.37	0.80	37.62
RF	WM	40	79	163.0	0.62	0.73	5.52	16.59	23.08	0.75	39.67	5.24	1.61	0.58	41.75
KA	OM	32	82	336.0	0.18	0.27	2.64	20.07	32.56	0.35	52.63	10.69	0.94	0.18	30.77
WC	OM	24	56	475.0	0	0.23	2.67	26.32	32.50	1.35	58.82	4.42	1.36	0.32	26.77
JH	WF	18	87	141.0	0.01	0.70	3.08	16.86	23.22	0.56	40.08	6.93	6.32	0.61	39.60
AF	WF	35	85	206.0	0.09	0.97	1.92	17.62	27.67	0.66	45.29	6.26	8.24	0.67	34.00
JJa	WF	22	90	132.0	0.07	0.20	3.22	22.74	27.58	0.58	50.32	6.04	1.04	0.65	35.06
AP	WF	22	81	127.0	0.06	10.76	2.93	16.72	22.86	0.38	39.58	5.90	6.63	0.41	31.26
Mean					0.09	1.19 (0.39)†	2.92	18.99	26.60	0.60	45.59	6.97	2.53 (1.17)‡	0.58	36.84
Standard deviation					±0.16	±2.78 (±0.29)†	±0.87	±2.86	±3.45	±0.31	±5.92	±1.87	±2.53 (±0.29)‡	±0.18	±5.06
Insulin independent diabetics															
RO	NM	44	113	51.4	0	0.41	2.57	11.63	10.76	1.03	22.39	5.46	3.09	1.34	59.43
CS	NM	50	79	343.0	0	0	2.02	14.24	16.43	0.41	30.67	14.76	1.30	0.74	48.35
JL	NM	36	93	116.0	0.06	0.33	1.54	10.78	21.46	0.61	32.24	5.05	18.67	1.36	37.23
AH	WM	49	187	380.0	0	0.10	3.04	17.27	24.68	0.16	41.95	7.01	0.63	0.55	45.12
TR	WM	40	83	183.0	0.19	0.74	4.42	22.55	27.94	0.29	50.49	10.33	1.18	0.68	29.56
NT	OM	35	89	83.0	0.10	0.68	2.92	14.21	22.72	0.61	36.93	8.00	1.64	0.98	45.52
LB	NF	60	129	252.0	0.41	0.60	2.22	15.98	17.98	0.67	33.96	7.77	2.11	0.44	42.60
BB	NF	68	111	47.3	0	0.02	1.94	10.38	16.69	0.55	27.07	8.46	1.92	0.66	56.12
AW	NF	41	195	505.0	0.08	0.10	2.14	13.02	11.73	0.83	24.75	8.53	1.50	0.88	57.58
EP	WF	60	179	113.0	0.79	0.90	2.24	20.60	30.29	1.43	50.89	5.00	3.97	1.96	22.94
Mean					0.16	0.39	2.51	15.07	20.07	0.66	35.13	8.04	3.60	0.96	44.45
Standard deviation					±0.24	±0.31	±0.77	±3.87	±6.18	±0.35	±9.48	±2.77	±5.11	±0.44	±11.36
p value					NS	NS	NS	.01-.02	.005-.01	NS	.005-.01	NS	NS	.01-.02	.05

*Fatty acids with retention time corresponding to arachidic acid (20:0) or greater.
†Mean of twelve controls exclusive of AP.
‡Mean of ten control values exclusive of AP, JH, AF.
NS = Not significant

due partially to insulin, is not due only to insulin since most of this "ILA" does not disappear after pancreatectomy.[23]

It appears that relatively little is known about the effect of "insulin-like" activity of plasma from diabetic subjects on formation of fatty acids. Our own data suggest that some mildly hyperglycemic insulin independent diabetics (FBS less than 150 mg. per 100 ml.) stimulate less, rather than more, incorporation of glucose 1-C-14 into fatty acids.[24] Therefore, these findings might after all be due to insulin deficiency — either relative or absolute.

Since we do not know the size of the various intermediary pools involved in formation of these fatty acids, we can draw no conclusions about the absolute or even relative amounts of the different fatty acids synthesized by these cells. However, it is important to realize that each mole of palmitate has approximately four to eight times as much radioactivity as each mole of 20 carbon fatty acid. Therefore, minor decreases in C-14 in palmitic acid and minor increases in C-14 in 20 carbon fatty acids could greatly distort the molar ratio of these fatty acids to each other.

These findings emphasize the multiplicity of abnormalities in fatty acid metabolism which may exist in diabetes. It appears to be important to consider

not only how much fatty acid is present or how many disintegrations of a radioactive substrate it contains, but also what kind of fatty acid is formed, the pathway by which it is formed, and the traffic this pathway will bear. In addition, the distribution and turnover of fatty acids in the various major lipid fractions should be known before even the fatty acid abnormalities in diabetes can be considered to be corrected.

In spite of the difficulties in interpretation and the difficulties in explanation of our findings, we can, nevertheless, state with reasonable certainty that an abnormality in formation of fatty acids, affecting primarily those made by de novo synthesis, and particularly palmitic acid, can be demonstrated in whole blood and platelets from insulin independent as well as insulin dependent diabetics. It may be important to consider the possible relationship of the abnormalities in platelet lipid formation to the vascular complications of diabetes.

It is also important to determine when, in the course of diabetes, these abnormalities develop. We are at present attempting to determine if they can be detected prior to the development of abnormal glucose tolerance.

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