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Abnormalities in Carbohydrate Tolerance Associated with Elevated Plasma Nonesterified Fatty Acids *

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Several abnormalities of carbohydrate metabolism common to a variety of endocrine and nutritional disorders have been shown recently to be associated with a high plasma concentration of nonesterified fatty acids (NEFA). For example, starvation or carbohydrate deprivation in the normal individual produces not only a marked impairment of carbohydrate tolerance, but also results in elevated levels of plasma NEFA (1–3). The coincident development of impaired carbohydrate tolerance and decreased sensitivity to insulin in association with elevated fasting plasma NEFA levels is seen in obesity (4–6), maturity-onset diabetes mellitus (7–9), acromegaly (5, 10, 11), pregnancy (12, 13), and subjects given exogenous human growth hormone (14–17). Randle, Garland, Hales, and Newsholme (18) have recently suggested that elevated plasma NEFA levels may be causally related to the impaired carbohydrate tolerance and decreased insulin sensitivity seen in these conditions, and have proposed the term "glucose-fatty-acid cycle" to denote the interactions between glucose and fatty acid metabolism in peripheral tissues responsible for the control of the blood glucose and fatty acid levels.

Considerable evidence from *in vitro* studies in several laboratories (18–22) can be marshalled in support of this intriguing concept, but to date there has been no demonstration that the circulating level of plasma NEFA influences either the rate of glucose utilization or the insulin responsiveness of the intact organism.[1] The present study was undertaken to determine whether an acute and sustained elevation of the plasma nonesterified fatty acid level in man can, in itself, impair carbohydrate tolerance and decrease the sensitivity of the peripheral tissues to insulin.

Methods

Experimental procedure. A simple experimental technique has been devised that rapidly raises the plasma NEFA level for a prolonged period and does not require the use of either endocrine or nutritional influences, which in themselves impair carbohydrate tolerance. The experimental procedure, hereafter referred to as the fat meal-heparin regimen, is based on the following observations: *1*) marked chylomicronemia develops 3 to 5 hours after a fat meal (23); *2*) chylomicrons are substrates for tissue lipoprotein lipase (24); and *3*) intravenous heparin activates lipoprotein lipase and its release into the circulation (24, 25). The fat meal-heparin regimen used in this study consisted of the ingestion of a 60-g fat meal of emulsified corn oil (60 g corn oil, 60 ml water, 15 g egg white, 6 ml vanilla extract, 0.5 g salt, and 0.4 ml sodium cyclamate) followed in 3 hours by the intravenous administration of 50 mg of heparin sodium.

Twelve normal subjects and five patients with mild diabetes mellitus according to the criteria of Fajans and Conn (26) were studied. None of the diabetic subjects required insulin therapy. Each individual acted as his own control since he was tested after an overnight fast with and without the fat meal-heparin regimen on one or more occasions. All subjects were on diets containing at least 200 g of carbohydrate for several days before testing. Carbohydrate tolerance was measured with the rapid intravenous glucose tolerance test, i.e., 25 g glucose intravenously over 4 minutes. The slope of the glucose disappearance curve when plotted as a semilogarithmic function represents the rate of glucose disappearance

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[1] While this manuscript was in preparation, Felber and Vannotti [Med. exp. (Basel) 1964, **10**, 153] published data demonstrating the impairment of glucose tolerance after the intravenous infusion of a fat emulsion.

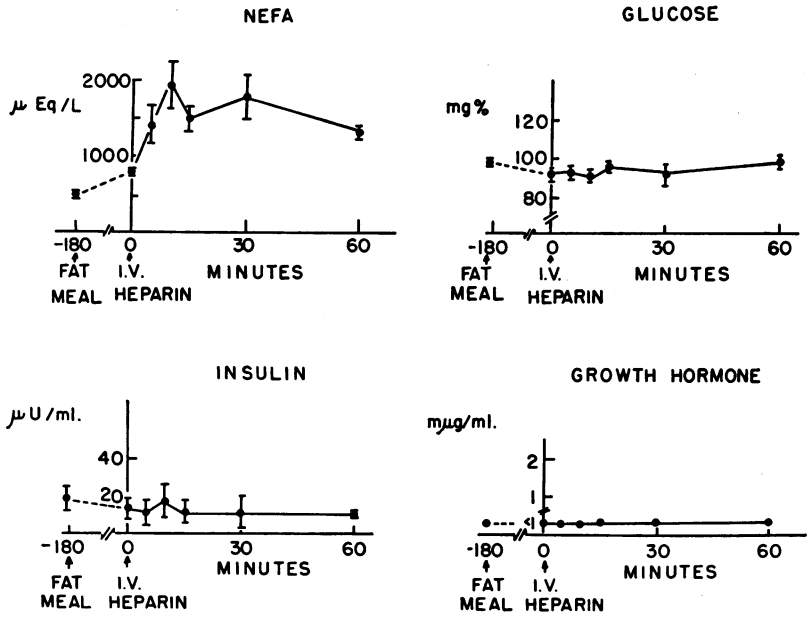

FIG. 1. EFFECT OF THE FAT MEAL-HEPARIN REGIMEN ON PLASMA NONESTERIFIED FATTY ACIDS (NEFA), GLUCOSE, INSULIN, AND GROWTH HORMONE LEVELS IN THREE NORMAL SUBJECTS. Each value represents the mean ± SEM.

(K) in per cent per minute (27). K can therefore be determined by the following formula:

$$K = \frac{\ln BG_1 - \ln BG_2 \times 100}{t_2 - t_1},$$ which reduces to

$$K = \frac{0.693}{t_{\frac{1}{2}}} \times 100,$$

where BG_1 = blood glucose at time$_1$, BG_2 = blood glucose at time$_2$, and $t_{\frac{1}{2}}$ = time when $BG_1/BG_2 = 0.5$. To assess the reproducibility of this method, repeat base-line studies were performed on seven normal subjects and showed an average variability of 13% between duplicate K rate determinations. All subjects were exposed to approximately the same duration of carbohydrate deprivation overnight before the control studies and when tested with the fat meal-heparin regimen. In the fat meal-heparin studies, the iv glucose tolerance test was started 15 minutes after the injection of heparin.

Analytical procedures. Glucose was determined in whole blood by the ferricyanide method with an Autoanalyzer, and plasma NEFA was determined by the microcolorimetric method of Duncombe (28) using the Dole extraction procedure (1). Since lipolysis continues *in vitro* after the intravenous administration of heparin, blood samples were rapidly cooled after collection and centrifuged for 5 minutes at 4° C, and 0.5 ml of plasma was added immediately to the extraction solution. The validity of this rapid extraction procedure was demon-

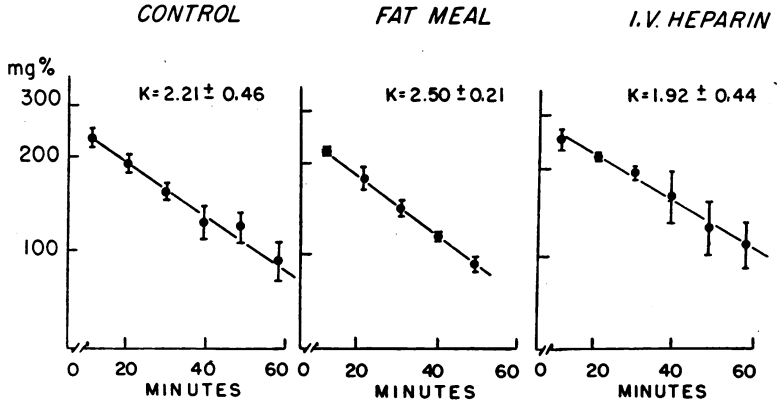

FIG. 2. EFFECT OF EITHER A FAT MEAL OR INTRAVENOUS HEPARIN SODIUM ON GLUCOSE TOLERANCE IN THREE NORMAL SUBJECTS. Each value represents the mean ± SEM. K = the rate of glucose disappearance.

TABLE I

Intravenous glucose tolerance

Subject Age, Sex	Minutes:	Control 0	8	16	24	32	40	48	56	64	$t_{\frac{1}{2}}$	K_e†
											minutes	
G.C.	Glucose*	76	233	214	175	155	151	122	121	94	42	1.65
38 M	NEFA	553	753	683	417	463	465	622	899	862		
	Insulin	9	45	55		42		40	25	37		
M.H.	Glucose	67	192	95	63	44	42	39	42	48	11	6.30
29 M	NEFA	402	697	463	546	398	506	566	480	582		
	Insulin	8	42	12	16	8	11	9	12	9		
T.H.	Glucose	78	284	147	103	93	84	70	70	64	12	5.77
31 M	NEFA	771	817	639	804	543	476	305	530	553		
	Insulin	12	42	39	32	15	14	17	9	14		
K.K.	Glucose	88	272	204	170	134	107	83	73	65	22	3.15
22 F	NEFA	720	773	490	370	304	331	331	423	330		
	Insulin	4	30	42	38	36	32	10	9	8		
G.L.	Glucose	88		227	142	128	108	100	92	87	25	2.77
25 M	NEFA	915	766	407	412	359	386	476	420	401		
	Insulin	11	31	38	27	24	23	12	14	19		
A.N.	Glucose	78	194	157	143	118	104	96	90	83	32	2.17
28 M	NEFA	673	623	563	531	447	418	265	261	302		
	Insulin	11	37	15	22	25	14	22	17	19		
J.P.	Glucose	74	213	172	145	97	101	76	80	78	26	2.67
21 M	NEFA	617	476	484	471	532	675	487	479	393		
	Insulin	26	64	33	19	27	11	24	17	17		
C.R.	Glucose	76	247	148	132	109	103	90	88	77	20	3.46
38 F	NEFA	725	770	862	554	431	493	541	496	388		
	Insulin	4	74	34	36	15	11	10	24	10		
C.S.	Glucose	58	281	172	138	124	112	99	90	84	20	3.46
22 F	NEFA	1,200	912	508	508	508	559	487	519	545		
	Insulin	7	39	31	28	27	14	24	12	7		
J.S.	Glucose	86	255	198	150	131	116	91	90	63	26	2.67
54 M	NEFA	761	761	606	511	542	545	425	425	431		
	Insulin	19	42	54		25		23	28	23		
S.S.	Glucose	90	320	232	226	194	161	142	123	104	36	1.92
28 F	NEFA	660	892	620	475	488	454	450	423	367		
	Insulin	18	57	42	47	46	51	53	38	15		
C.W.	Glucose	78	242	210	155	147	121	111	103	82	32	2.17
31 M	NEFA	1,266	1,325	1,053	742	681	681	548	564	564		
	Insulin	3	38	38	40	30	20	24	40	10		
Glucose	Mean	78	248	181	145	123	109	93	89	77	25.3	2.74
	SEM	±3	±12	±12	±11	±11	±5	±8	±6	±4	±1.3	±0.14
NEFA	Mean	772	797	616	528	475	499	459	493	477		
	SEM	±72	±58	±51	±37	±29	±30	±32	±43	±45		
Insulin	Mean	11	45	36	28	27	18	22	20	16		
	SEM	±2	±4	±4	±4	±3	±4	±2	±3	±2		

* The units of measurement are: glucose, mg per 100 ml; nonesterified fatty acids (NEFA), μEq/L; insulin, μU/ml.
† K_e = glucose disappearance rate during control studies (per cent × minute^{-1}).
‡ K_e = glucose disappearance rate during fat meal-heparin studies (per cent × minute^{-1}).

strated in the following manner: postheparin blood specimens from six subjects were each collected in two test tubes, one of which contained SAP-36,[2] a polyanion inhibitor of lipoprotein lipase (29). When the rapid extraction technique was used, the average NEFA value was 1,007 ± 125 μEq per L (standard error of the mean) for plasmas containing SAP-36, and 1,187 ± 191 μEq per L for plasmas without. This difference is statistically in-

[2] SAP-36 is a corn amylopectin kindly supplied by Dr. Peter Bernfeld, Bio-Research Institute, Cambridge, Mass.

TABLE I

tests in normal subjects

Fat meal—heparin													
−180	−15	0	8	16	24	32	40	48	56	64	$t_{\frac{1}{2}}$	K_e‡	$\frac{K_e}{K_e} \times 100$
												minutes	
94	94	98	234	231	226	191	180	155	163	141	81	0.86	52
447	690	1,670	1,604	995	958	775	742	813	597	478			
18	20	18	54	40	40	42	36	33	45	44			
90	87	99	185	124	103	89	80	75	73	75	21	3.30	52
565	717	1,990	1,737	1,345	1,086	816	839	850	837	903			
	27	18	80	45	27	22	20	21	16	14			
83	79	76	203	151	123	106	90	80	78	77	27	2.57	45
584	1,030	2,310	1,804	1,830	1,278	1,383	1,614	1,185	1,250	1,338			
9	11	8	80	64	30	32	12	19	13	8			
79	78	80	232	197	176	155	140	127	118	107	49	1.41	45
726	1,205	2,506	1,471	1,737	962	883	510	466	633	466			
9	2	2	36	40	44	24	22	12	14	6			
98	86	86		180	139	118	97	83	85	75	28	2.48	90
729	869	1,255	878	769	801	801	809	862	795	729			
13	4	6	85	52	36	22	20	17	6	3			
84	70		208	127	119	110	97	90	82	78	41	1.69	78
563	1,456		2,412	2,159	1,538	1,307	1,062	1,149	922	925			
13	8	21	39	18	14	11	12	16	9	13			
94	82	92	203	179	143	123	115	82	84	90	37	1.87	70
407	638	1,718	1,386	1,452	1,229	854	854	734	734	558			
9	19	18	57	42	36	32	28	26	15	15			
71	72	73	232	173	139	118	84	76	66	66	24	2.89	84
762	872	1,990	1,428	1,561	999	1,021	753	816	755				
4	10	6	90	38	35	28	24	25	20	22			
80	71	68	290	176	128	97	89	70	66	54	18	3.85	111
992	1,452	1,931	1,599	1,122	888	904	906	763	774	774			
11	8	6	91	95	53	38	18	19	17	10			
99	89	95	226	210	200	164	137	145	143	120	57	1.18	44
403	807		1,630	1,431	1,165	1,108	835	810	681	624			
29	19	22	93	70	78	65	45	47	40	28			
93	104	98	268	262	234	171	195	186	166	150	68	1.02	53
534	810		2,140	1,201	747	726	603	742	857	624			
29		10	45	35	18	63	63	70	47	55			
89	80	78	249	188	155	121	92	77	67	64	23	3.02	139
948	1,067	1,644	1,444	1,418	1,161	888	775	710	532	557			
15	12	2	40	34	10	15	24	22	10	9			
88	83	79	230	183	157	130	116	104	99	91	39.5	1.75	
±2	±3	±3	±9	±11	±12	±9	±11	±11	±11	±9	±5.9	±0.27	
589	926	1,840	1,628	1,418	1,068	956	859	825	781	725			
±48	±85	±108	±109	±109	±64	±61	±80	±55	±54	±77			
14	13	11	66	47	35	33	27	27	22	19			
±2	±2	±2	±7	±6	±4	±5	±4	±5	±4	±5			

significant ($p > 0.2$) and demonstrates that very little *in vitro* lipolysis occurs during the rapid extraction procedure.

Insulin was assayed immunologically by a modification of the double antibody method of Morgan and Lazarow (30). This modification consists of using a 72-hour incubation period for the initial antigen-antibody interaction and using a rabbit anti-guinea pig gamma-globulin serum for precipitating the insulin antibody complex. Human growth hormone was determined by the

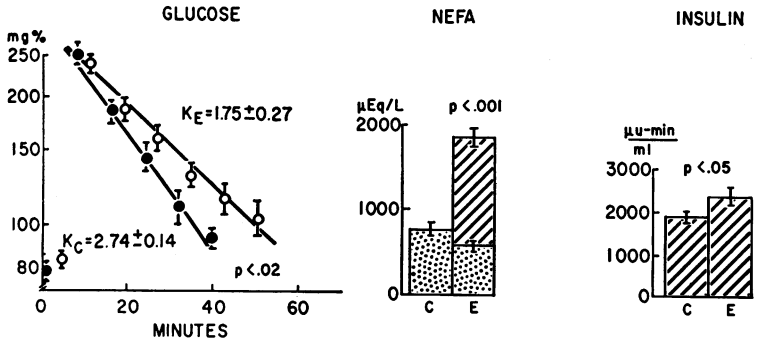

FIG. 3. EFFECT OF THE FAT MEAL-HEPARIN REGIMEN ON GLUCOSE DISAPPEARANCE, PLASMA NEFA, AND INSULIN SECRETION IN NORMAL SUBJECTS. ● = control study (C); ○ = fat meal-heparin regimen (E); I = SEM. Rate constants (K) are expressed as per cent × minutes^{-1} ± SEM. The fasting plasma NEFA levels are represented by stippled bars, and the elevation after the fat meal-heparin regimen by the cross-hatched bar above.

radioimmunoassay method of Schalch and Parker (31). Plasmas assayed for insulin and human growth hormone were stored at −20° C until used. Since pancreatic insulin secretion could not be measured directly in these studies, the area circumscribed by the plasma insulin response curve has been used as an index of insulin secretion and is expressed as microunit-minutes per milliliter.

Results

Effect of the fat meal-heparin regimen on plasma levels of NEFA, glucose, insulin, and growth hormone. Three normal subjects were studied on two or more occasions after an overnight fast to determine the effect of the fat meal-heparin regimen on plasma NEFA, glucose, insulin, and growth hormone (Figure 1). Three hours after the ingestion of the fat meal, the plasma NEFA content rose from an average fasting level of 488 ± 45 μEq per L to 767 ± 11 μEq per L. After the intravenous injection of 50 mg of heparin sodium, the plasma NEFA level increased rapidly to 1,929 ± 274 μEq per L and remained greater than 1,300 μEq per L for over an hour. Throughout this period, the plasma levels of glucose and insulin remained unchanged, and the level of growth hormone remained less than 1 mμg per ml.

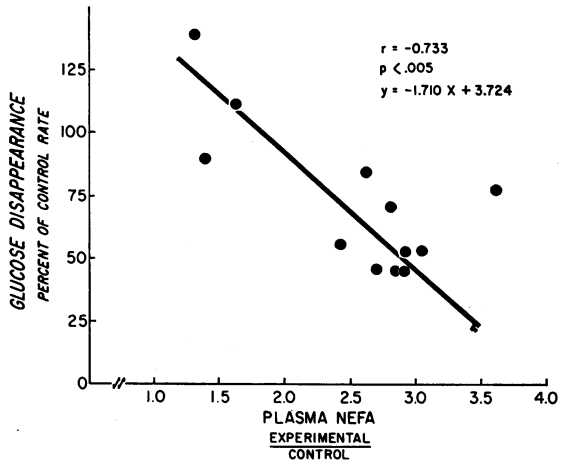

FIG. 4. CHANGE IN RATE OF GLUCOSE DISAPPEARANCE IN RELATION TO THE INCREASE IN PLASMA NEFA.

Effect of either a fat meal or intravenous heparin on carbohydrate tolerance. The glucose disappearance rate did not change significantly from control values when measured in three normal subjects on two or more occasions either 3 hours after the ingestion of a fat meal alone or 15 minutes after the intravenous administration of 50 mg heparin sodium (Figure 2). In these individuals, the plasma NEFA level increased from an average fasting level of 502 ± 73 μEq per L to 674 ± 177 μEq per L after the fat meal. After intravenous heparin alone the increase in plasma NEFA was from 597 ± 79 μEq per L to 970 ± 167 μEq per L, but the rise was transient with a return to normal fasting levels within 10 to 15 minutes. Plasma insulin response during these studies did not differ significantly from that seen under control conditions (control 1,617 ± 112, fat meal 1,168 ± 154, heparin 1,147 ± 152 μU-minutes per ml.

Effect of fat meal-heparin regimen on carbohydrate tolerance in normal subjects. The carbohydrate tolerance in twelve normal subjects was

studied under both control conditions and after the administration of a fat meal and heparin regimen (Table I). The fat meal-heparin regimen resulted in a marked reduction (> 45%) in carbohydrate tolerance in six subjects, a moderate reduction (> 16%) in three others, and no reduction in the remaining three subjects (G.L., C.S., C.W.). When compared to the mean control K value, the average decrease in the glucose disappearance rate after the fat meal-heparin regimen was 36.1%, dropping from a mean base-line value of 2.74 ± 0.14 to a mean experimental value of 1.75 ± 0.27% per minute (Figure 3). This decrease is significant with a p value < 0.02. All three subjects that showed no reduction (two actually showed an increase) in the glucose disappearance rate during the fat meal-heparin study had markedly elevated fasting plasma NEFA levels on the day of the control study (915, 1,200, 1,266 μEq per L). The average fasting plasma NEFA level during the control studies for the twelve normal subjects was 772 ± 72 μEq per L. During the experimental studies the average fasting plasma NEFA level of 589 ± 48 μEq per L showed a significant rise to 1,840 ± 108 μEq per L (p < 0.005) after the administration of a fat meal and intravenous heparin. The average insulin secretion (as previously defined) in response to 25 g of iv glucose was 1,878 ± 159 μU-minutes per ml during the control studies, and increased significantly (p < 0.05) to 2,352 ± 231 μU-minutes per ml during the fat meal-heparin studies.

The degree of impairment of glucose tolerance in these normal subjects closely paralleled the increase in plasma NEFA level. The regression line, plotted by the method of least squares, relating the glucose utilization rate seen after the fat meal-heparin regimen and the corresponding increase in plasma NEFA level is recorded in Figure 4. The coefficient of correlation (r) equals − 0.733 and is significant with a p value of < 0.005.

Effect of fat meal-heparin regimen on carbohydrate tolerance in mild diabetes mellitus. Similar studies were performed on five mild diabetics who were controlled on diet alone and had normal fasting blood sugar and plasma NEFA levels (Table II). During the control studies, the average glucose disappearance rate of the diabetic group was 1.24 ± 0.16% per minute (Figure 5), approximately 45% of the control value for normal subjects. After the fat meal-heparin regimen, the mean plasma NEFA level rose from the fasting value of 578 ± 84 μEq per L to 2,101 ± 471 μEq per L, but there was no further impairment in carbohydrate tolerance (K = 1.24 ± 0.18). It should be noted that the reduced glucose disappearance rate in normal individuals on the fat-heparin regimen (K = 1.75 ± 0.27) approaches the disappearance rate in these diabetic subjects. The plasma insulin response in diabetic patients during the control study was 1,861 ± 417 μU-minutes per ml, only slightly less than that seen in normal individuals, and it was not significantly altered during the fat meal-heparin period (2,318 ± 167 μU-minutes per ml).

Temporal relationship between the fatty acid mobilizing activity and the insulin antagonistic effect of growth hormone. Since these studies indicate that an increase in the level of circulating NEFA may be associated with impaired carbohydrate tolerance and insulin responsiveness, the temporal relationship between the fatty acid mobilizing activity of human growth hormone (HGH) and its well-known insulin antagonistic action was explored. After initial base-line studies, repeat intravenous glucose tolerance tests were performed on seven normal subjects on different days at 10, 60, and 120 minutes after the intravenous administration of 5 mg of human growth hormone (Table III). Ten minutes after the administration of growth hormone (Figure 6), its acute "insulin-like effect" produced an average increase of 32% in the glucose disappearance rate over the mean control value for the same subjects (p < 0.005). Sixty minutes after HGH administration, the average glucose disappearance rate returned to approximately the control value. The average plasma NEFA levels 10 and 60 minutes after HGH administration were insignificantly different from the mean control value. One hundred twenty minutes after the administration of HGH, the glucose disappearance rate decreased 48% from the control value (p < 0.005) while at the same time the plasma NEFA level rose 103% over the mean fasting value (p < 0.005).

Discussion

The results of this study support the proposal of Randle and his associates (18) that the circu-

TABLE II

Intravenous glucose tolerance

Subject Age, Sex	Minutes	Control 0	8	16	24	32	40	48	56	64	$t_{\frac{1}{2}}$	K_c
											minutes	
F.B.	Glucose*	88	254	220	195	167	165	150	137	116	52	1.33
51 M	NEFA	572	614	593	489	534	500	468	521	383		
	Insulin	7	19	15	10	18	10	6	15	14		
M.G.	Glucose	77	265	237	238	217	202	173	182	174	83	0.84
50 F	NEFA	510	696	498	673	408	304	330	438	338		
	Insulin	4	8	9	14	30	30	22	10	12		
L.H.	Glucose	75	234	194	171	148	137	114	106	98	40	1.73
20 F	NEFA	450	412	490	344	490	440	402	459	419		
	Insulin	13	53	42	38	40	34	16	33	37		
W.J.	Glucose	96	308	266	214	208	194	176	158	144	48	1.44
56 F	NEFA	707	846	691	657	538	657	451	728	799		
	Insulin	25	36	44	52	40	48	48	46	46		
B.W.	Glucose	94	226	188	173	167	155	150	132	118	59	1.18
23 M	NEFA	532	614	561	534	588	540	866	417	372		
	Insulin	16	41	26	30	32	29	26	30	16		
Glucose	Mean	86	257	221	198	181	171	153	143	130	56	1.24
	SEM	±5	±14	±14	±13	±13	±12	±11	±13	±13	+7	±0.17
NEFA	Mean	554	636	567	539	512	488	503	513	462		
	SEM	±43	±70	±36	±60	±30	±58	±94	±57	±85		
Insulin	Mean	13	31	37	29	32	28	24	27	23		
	SEM	±4	±8	±7	±8	±4	±6	±7	±6	±5		

* The units of measurement are: glucose, mg per 100 ml; NEFA, μEq per L; insulin, μU per ml.

lating level of nonesterified fatty acids may be an important factor in regulating the glucose tolerance and insulin responsiveness of the intact organism. The rapid intravenous glucose tolerance test, used in this study for assessing glucose disappearance rates in the total organism, does not permit conclusions regarding the changes induced by the fat meal-heparin regimen on the metabolism of glucose by specific organ systems, in particular, striated muscle, adipose tissue, and liver. On the basis of *in vitro* studies, however, increased NEFA concentrations would be expected to decrease the

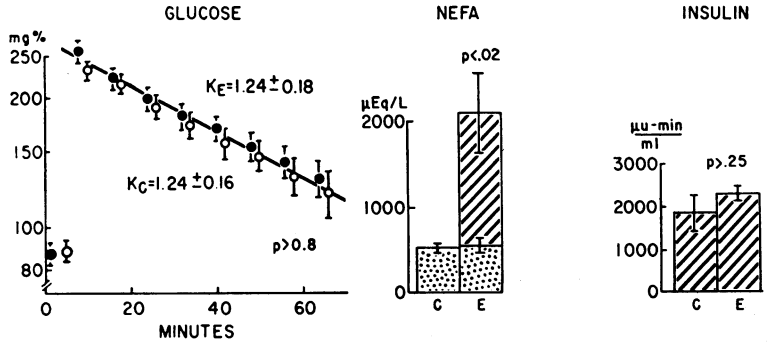

FIG. 5. EFFECT OF THE FAT MEAL-HEPARIN REGIMEN ON GLUCOSE DISAPPEARANCE, PLASMA NEFA, AND INSULIN SECRETION IN SUBJECTS WITH MILD DIABETES MELLITUS. ● = control study (C); ○ = fat meal-heparin regimen (E); I = SEM. Rate constants (K) are expressed as per cent × minutes^{-1} ± SEM. The fasting plasma NEFA levels are represented by stippled bars, and the elevatioin after the fat meal-heparin regimen by the cross-hatched bar above.

TABLE II

tests in diabetic subjects

Fat meal—heparin													
−180	−15	0	8	16	24	32	40	48	56	64	$t_{\frac{1}{2}}$	K_e	$\frac{K_e}{K_c}\times 100$
											minutes		
100	83	95	208	194	168	145	129	136	117	105	56	1.24	93
798	1,072	2,456	2,317	2,783	2,104	1,966	1,186		1,431	1,540			
21	12	13	40	29	34	35	28	30	25	24			
78	90	100	249	246	222	207	196	180	168	154	78	0.89	106
761	2,091	3,727	2,383	1,559	1,681	1,293	1,399	832	939	755			
15	18	19	39	33	23	26	25	43	34	48			
84	61	88	243	218	193	178	166	156	147	136	57	1.22	71
397	798	1,930	2,170	1,777	1,578	1,372	1,219	1,095	1,000	842			
18	24	7	64	33	38	35	33	34	34	30			
122	78	75	253	227	210	192	180	158	141	130	59	1.17	81
443	578	1,205	938	853	716	532	841	596	705	759			
20	23	8	26	35	48	58	46	28	30	25			
84		82	199	190	159	138	121	107	88	74	40	1.73	147
489	558	1,189	1,053	979	816	851	670	681	747	854			
22	25	11	60	34	41	30	30	28	15	22			
94	78	88	230	215	190	172	158	147	132	120	58	1.24	100
±8	±6	±5	±12	±10	±12	±13	±14	±12	±14	±16	±6	±0.18	±13
578	1,019	2,101	1,772	1,590	1,379	1,203	1,023	801	964	950			
±84	±284	±471	±319	±266	±266	±244	±113	±109	±129	±149			
19	20	12	46	33	37	37	32	33	28	30			
±1	±2	±2	±6	±2	±4	±6	±4	±3	±4	±5			

rate of glucose utilization by striated muscle and impair the sensitivity of this tissue to insulin (19–22). Adipose tissue, on the other hand, might conceivably respond in a different manner. Leboeuf and Cahill (32) have reported that increased levels of nonesterified fatty acids stimulate glucose uptake, glucose oxidation to CO_2, and glucose conversion to glyceride-glycerol by the rat epididymal fat pad preparation *in vitro*. The similar effects of fatty acids, epinephrine, ACTH, and growth hormone on glucose metabolism of adipose tissue have led these investigators to suggest that the hormone-induced changes in glucose utilization in this tissue are secondary to their lipolytic activity. Although every precaution was taken to perform the control and fat meal-heparin studies under as comparable conditions as possible, it is readily acknowledged that individual differences in the rate of release of endogenous epinephrine during these studies may have produced some of the variability observed in the glucose disappearance rates in the normal subjects during periods of normal and elevated plasma NEFA levels. The effect of fatty acids on hepatic glucose metabolism has not been systematically studied, but it has been reported that the intravenous infusion of sodium octanoate at a rate sufficient to produce a significant ketonemia did not affect net splanchnic glucose production (33).

The temporal correlation between the appearance of increased plasma NEFA levels and decreased glucose disappearance rates that follow the intravenous injection of human growth hormone is consistent with the concept that the insulin antagonistic effect of growth hormone is secondary to its lipolytic activity. The severity of impairment of glucose tolerance after growth hormone administration, however, is greater than would have been predicted from the plasma NEFA level, using as a basis of comparison the relationship between acute elevation in plasma NEFA level and the associated decrease in glucose disappearance rate seen after the fat meal-heparin regimen (Figure 4). This apparent discrepancy could be accounted for if the intracellular rather than the extracellular concentration of free fatty acids is the significant factor influencing glucose metabolism. In this context, raising the extracellular free fatty

TABLE III

Intravenous glucose tolerance tests in normal subjects

Subject	Minutes:	NEFA 0*	Control Glucose 0*	5	10	15	20	25	30	40	$t_{\frac{1}{2}}$	K†
		μEq/L				*mg per 100 ml*					*minutes*	
G.C.		553	91	242	236	214	201	178	153	132	45	1.54
H.D.		614	93	248	217	203	183	171	165	136	49	1.41
J.F.		537	72	262	222	184	152	132	98	78	20	3.46
D.H.		479	108	267	190	117	128	105	81		20	3.46
E.M.		844	76	297	260	191	157	138	124	100	20	3.46
J.S.		596	86	255	198		150	131	116	91	26	2.67
D.T.		660	86	246	212	196	178	174	158	128	42	1.65
Mean		612	87.4	260	219	184	164	147	128	111	31.7	2.19
SEM		±44.5	±4.5	±7.1	±8.9	±14.1	±9.3	±11.9	±12.1	±10	±4.9	±0.35
							60 Minutes After HGH					
G.C.		449	86	237	226	214	196	190	162	138	44	1.58
H.D.		516	83	227	197	183	177	173	163	153	68	1.02
J.F.		612	91	247	204	188	170	155	130	103	27	2.57
D.H.		680	97	255	200	172	144	140	116	92	23	3.01
J.S.		548	106	284	217	196	169	160	145	102	29	2.39
D.T.		259	87	223	195	167	144	140	128	120	32	2.16
Mean		511	97.7	246	216	187	167	160	141	118	37.2	1.86
SEM		±59.9	±3.5	±9.1	±5.0	±6.9	±8.2	±7.9	±7.9	±9.6	±6.8	±0.35

* Zero minutes denotes the time immediately before the intravenous administration of 25 g of glucose.
† K = glucose disappearance rate (per cent × minute^{-1}).
‡ HGH = human growth hormone.

acid level, e.g., a fat meal-heparin regimen, appears to be less effective in increasing the intracellular fatty acid content than hormone-stimulated lipolysis. Consistent with this suggestion are the results of Verner, Blackard, and Engel (34), who demonstrated that epinephrine-induced lipolysis was not associated with increased glucose uptake in adipose tissue if the intracellular free fatty acid content was permitted to rise. Increased glucose utilization did occur, however, if a fatty acid ac-

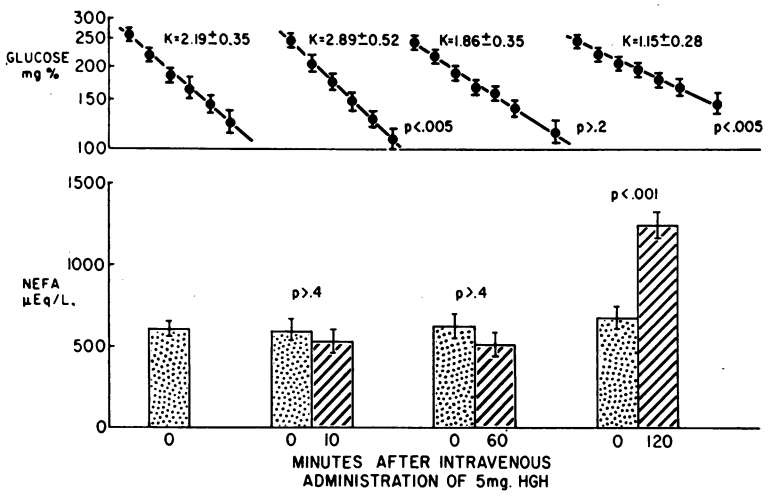

FIG. 6. EFFECT OF THE INTRAVENOUS ADMINISTRATION OF HUMAN GROWTH HORMONE ON GLUCOSE DISAPPEARANCE AND PLASMA NEFA. Average plasma NEFA values immediately before the administration of 5 mg human growth hormone (HGH) are represented by the stippled bars and before the intravenous administration of 25 g of glucose by the cross-hatched bars below each corresponding glucose disappearance curve.

TABLE III

after 5 mg of intravenous human growth hormone

NEFA 0*	10 Minutes After HGH‡ Glucose 0*	5	10	15	20	25	30	40	$t_{\frac{1}{2}}$	K
μEq/L				*mg per 100 ml*					*minutes*	
736	89	252	228	204	174	153	132	104	27	2.57
	82		204	188	164	155	148	144	46	1.51
412	68	226	172	159	125	106	70	70	18	3.85
277	108	267	190	117	128	105	81		14	4.95
530	99	290	234	189	148	124	90		15	4.62
667	96	207	183		146	124	104	90	21	3.30
	76	250	224	188	160	142	126	100	27	2.57
524	88.3	249	205	174	149	130	107	102	24.0	2.89
±83.4	±5.2	±12.0	±9.2	±12.9	±6.9	+7.8	±10.9	±12.1	±4.2	±0.52
					120 Minutes After HGH					
1,205	110	263	246	240	232	215	210	198	82	0.84
1,403	100	226	214	204	200	106	188	184	120	0.58
1,401	68	254	228	204	198	176	168	144	42	1.65
1,199	81	269	228	201	183	172	155	125	31	2.24
1,358	88	232	207	196	190	170	154	146	50	1.38
872	94	238	204	176	162	150	136	124	38	1.82
1,240	90.2	247	221	204	194	180	169	154	60.5	1.15
±82.6	±6.0	±7.2	±6.5	±8.5	±9.4	±9.2	±10.9	±12.6	±13.9	±0.28

ceptor, e.g., albumin, was added to the incubation medium to keep the intracellular fatty acid concentration low (32). Several recent studies have further indicated that it is not the fatty acids per se but rather the fatty acid acyl CoA derivatives that are directly responsible for the changes in enzyme activities resulting in the alterations in carbohydrate and lipid metabolism seen in conditions characterized by high plasma fatty acid levels (35–37).

Summary

A simple method has been described for producing an acute and sustained elevation of the plasma nonesterified fatty acid (NEFA) level. The results of this study indicate that in man an elevation in plasma NEFA concentration may be associated with an impaired glucose tolerance and decreased target organ sensitivity to insulin. The degree of impairment in carbohydrate tolerance is closely correlated with the elevation in the plasma NEFA level. After growth hormone administration the rise in plasma NEFA is temporally related to the appearance of insulin antagonism. These studies support the concept that several of the abnormalities of carbohydrate metabolism associated with growth hormone administration, starvation, pregnancy, obesity, and diabetes mellitus may be a consequence, at least in part, of the elevated nonesterified fatty acid levels characteristic of these conditions.

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