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ROLE OF FREE FATTY ACIDS IN FOREARM METABOLISM IN MAN, QUANTITATED BY USE OF INSULIN *

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Previous studies (1, 2) have demonstrated that glucose uptake by muscle of the forearm of man is inadequate to account for more than about 10 per cent of its observed oxygen consumption. Because forearm respiratory quotient (RQ) is approximately 0.7, which suggests combustion of long-chain fatty acids, it was predicted that the fraction of plasma lipid known as free fatty acids (FFA) would prove to be the missing substrate of forearm muscle. Extensive examination of this problem has, however, failed to demonstrate consistently positive arterio-deep venous (A – DV) FFA differences across the human forearm (2). There is evidence, based on isotopic studies by Friedberg, Klein, Trout, Bogdonoff, and Estes (3), that the human forearm does remove FFA from arterial blood, and the possible factors responsible for our failure to demonstrate this by simple measurement of A – DV concentration differences have been treated in detail elsewhere (2). In brief, while FFA is being extracted from plasma by forearm muscle, adipose tissue, intimately surrounding muscle, adds fatty acid to venous plasma. The net A – DV FFA difference is determined by the relative rates of these two processes.

The validity of this hypothesis could be tested if there were an agent that inhibited release of FFA from adipose tissue without also affecting FFA uptake by peripheral tissues. Insulin may be such an agent.

There is no doubt that insulin inhibits FFA release from adipose tissue, a fact demonstrated both *in vivo* by Gordon (4), Estes and associates (5), and Bierman, Schwartz, and Dole (6), and *in vitro* (7, 8). There is, however, conflicting evidence on whether or not insulin influences FFA uptake by peripheral tissues. In excised adipose tissue, in the presence of both glucose and insulin, FFA incorporation is increased (7). But there is no such evidence from experiments *in vivo*. Indeed, it is unproven that there is substantial FFA uptake by adipose tissue *in vivo* under any circumstances. Bragdon and Gordon (9) found only 1 to 2 per cent of injected palmitic acid-1-C^{14} in adipose tissue, a figure not increased by prior administration of glucose, which stimulates insulin secretion. It has been demonstrated by Spitzer and Hohenleitner in the dog (10) and by Scow, Robert, and Chernick (11) in isolated rat parametrial adipose tissue that, while net production of FFA by adipose tissue was inhibited by insulin, significant net uptake by adipose tissue was not produced. With respect to a possible effect of insulin on FFA uptake by muscle, experiments capable of testing this crucially *in vivo* have not been done. Absence of any such action, however, is suggested by the fact that turnover rates of FFA, measured by use of isotopes in the dog, are not changed by insulin (6, 12). Fritz (13) showed that oxidation of palmitic acid-1-C^{14} by excised rat diaphragm was not altered by insulin.

Thus, while it is not established that insulin may not influence FFA uptake by peripheral tissues, most of the available data suggest that it does not do so in the intact animal. In the light of previous studies showing that the human forearm is remarkably sensitive to local intra-arterial infusion of small concentrations of insulin (14), a technique was available to establish the effects of insulin, injected intra-arterially at a final concentration of several hundred microunits per milli-

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TABLE I

*Mean effect of insulin on forearm metabolism**

Time after insulin started	Plasma flow	Glucose		Lactate		FFA	
		A − DV	A − SV	A − DV	A − SV	A − DV	A − SV
min	*ml/min/100 ml arm*	*μmole/ml*		*μmole/ml*		*μmole/ml*	
0	2.0 ± 0.18	0.12 ± 0.016	0.17 ± 0.013	−0.09 ± 0.010	−0.15 ± 0.015	+0.02 ± 0.038	−0.16 ± 0.033
12	2.3 ± 0.22	0.48 ± 0.079	0.43 ± 0.048	−0.11 ± 0.010	−0.12 ± 0.011	+0.02 ± 0.022	+0.02 ± 0.054
18	2.4 ± 0.17	0.88 ± 0.086	0.65 ± 0.061	−0.14 ± 0.020	−0.17 ± 0.029	+0.06 ± 0.021	+0.06 ± 0.016
26	2.8 ± 0.79	1.39 ± 0.128	0.83 ± 0.038	−0.18 ± 0.010	−0.23 ± 0.030	+0.08 ± 0.014	+0.06 ± 0.020
40	2.3 ± 0.29	1.28 ± 0.060	0.80 ± 0.032	−0.22 ± 0.010	−0.21 ± 0.035	+0.09 ± 0.033	+0.09 ± 0.023
60	2.0 ± 0.40	0.65 ± 0.084	0.50 ± 0.044	−0.21 ± 0.020	−0.24 ± 0.037	+0.02 ± 0.041	−0.03 ± 0.013
90	1.7 ± 0.25	0.54 ± 0.138	0.47 ± 0.057	−0.20 ± 0.030	−0.26 ± 0.041	−0.07 ± 0.051	−0.06 ± 0.042

* Insulin administered intra-arterially for 26 minutes beginning at time zero. Values given at time zero are means of all control measurements taken before insulin infusion started. Data for arterio-deep venous (A − DV) and arterio-superficial venous (A − SV) concentration differences are means of values obtained in 5 subjects, except for A − SV concentration differences obtained at 12 minutes, which are means of values in 4 subjects, and for A − DV and A − SV concentration differences obtained at 90 minutes, which are means of values in 3 subjects. All values are means ± SE of mean.

liter of brachial arterial plasma, on FFA metabolism in the human forearm. Information was sought concerning the following two problems. *1*) Basal arterio-superficial venous (A − SV) FFA concentration differences are almost invariably negative, thereby reflecting discharge of fatty acids from forearm adipose tissue into venous plasma. Insofar as A − SV concentration differences reflect metabolism of forearm adipose tissue, does insulin, under the conditions to be employed, in fact obliterate negative A − SV FFA concentration differences? *2*) What effect, if any, does insulin exert on A − DV FFA concentration differences and, if one assumes the complete oxidation of abstracted fatty acids, is sufficient FFA uptake unmasked to account for most of the oxygen consumption of skeletal muscle? Of course glucose uptake under maximal insulin stimulation is sufficient to account entirely for oxygen consumption by forearm muscle if all the glucose is oxidized. Previous studies, however, suggest that none of the glucose entering muscle under the influence of insulin is oxidized during the time of the acute experiment (14). A preliminary report of some of these observations has appeared (15).

METHODS

Five young adult subjects were studied. Dietary history of carbohydrate intake was normal. No food was taken after 8 p.m. on the evening preceding the experiment. Studies were performed between the hours of 9 a.m. and 1 p.m. A brachial artery, an ipsilateral antecubital vein, draining muscle but also undoubtedly contiguous adipose tissue, and a superficial vein, draining mostly but not exclusively forearm adipose tissue and skin, were cannulated by techniques described previously (2). Blood flow was measured by the indicator-dilution method, based on continuous injection at constant rate of the dye T-1824 (1). Two simultaneous sets of arterial, deep venous (DV), and superficial venous (SV) samples were collected at 15-minute intervals before the infusion of insulin.

Glucagon-free insulin[1] was then injected intra-arterially for 26 minutes at a rate delivering 100 μU of insulin per minute per kg body weight. Blood samples were drawn 12, 18, and 26 minutes from commencement of the infusion, and for periods up to an hour thereafter. Circulation to the wrist and hand was excluded for at least 5 minutes preceding collection of blood samples and for the entire period of insulin administration.

Net uptake or output of a metabolite by forearm muscle was calculated from the equation $\dot{Q} = F(A - DV)$, where $\dot{Q}$ is uptake or output of the metabolite in units of mass per minute per 100 ml of forearm flow, F is blood or plasma flow through the forearm in milliliters per minute per 100 ml of arm, and A and DV are concentrations of the metabolite in arterial and deep venous blood or plasma, respectively, in units of mass per milliliter. This equation overestimates $\dot{Q}$, since it assigns all of forearm blood flow to muscle. The errors have been discussed in detail elsewhere (1). Handling of blood and analytical methods were as previously described (2).

RESULTS

Results appear in Tables I and II.

Insulin concentration in forearm plasma. Calculated brachial arterial insulin concentration varied from 300 to 700 μU per ml, levels comparable to those achieved in an earlier study (14).

Plasma flow. Except for one subject, L, plasma flow remained acceptably constant; that is, it did

[1] Lot no. W 3606, Eli Lilly and Company, Indianapolis, Ind.

not vary by more than 20 per cent about the mean in any of the remaining four subjects.

Arterial concentrations. Arterial concentrations of glucose and lactate remained constant. We have previously shown that the arterial concentration of FFA is labile under basal postabsorptive conditions (2). Changes in arterial concentration of FFA appear to be related to the height of the initial values: low concentrations tend to rise and high ones to fall. In the light of these considerations, the course of arterial concentration of FFA in the present series may be considered not to have varied unusually (Figure 1). In particular, no consistent arterial hypolipacidemia was noted after insulin administration. In one subject, L, there was a 50 per cent rise in arterial FFA just before conclusion of the study.

Glucose (Figure 2). Insulin promptly increased both A − DV and A − SV glucose concentration differences, the former ten and the latter five times the resting value. Temporal events in the deep and the superficial forearm beds paralleled each other closely, with the maximal effect reaching a plateau from approximately 18 to 40 minutes and then decaying fairly rapidly.

Lactate. A small but definite increase in negative lactate A − DV and A − SV concentration differences occurred during and after insulin administration. Because glucose uptake increased more than lactate production, the fraction of glucose uptake accounted for by lactate production (L/G ratio) decreased from a basal value of greater than 0.4 to less than 0.2 in both forearm beds after insulin administration.

TABLE II

*Effect of insulin on respiratory quotient, metabolism (Q) of O_2 and CO_2, and FFA/O_2, of forearm muscle**

	Basal effect	Peak insulin effect
Forearm $\dot{Q}O_2$, *μmoles/min/100 ml arm*	9.36 ± 1.27	9.2 ± 1.15
Forearm $\dot{Q}CO_2$, *μmoles/min/100 ml arm*	6.32 ± 1.02	6.27 ± 0.78
Respiratory quotient	0.70 ± 0.04	0.70 ± 0.06
FFA/O_2		
Subject S	0.65	0.65
L	0.48	0.60
B	0.17	0.42
Y	0.09	0.42
E	−0.77	0.69
Mean	0.12 ± 0.24	0.56 ± 0.09

* FFA/O_2 = fraction of oxygen consumption accounted for by FFA oxidation. For calculation see text. Each of the 5 subjects is identified by an initial. Other values are means ± SE of mean.

A − SV FFA differences (Figure 3). Under basal conditions, negative A − SV FFA differ-

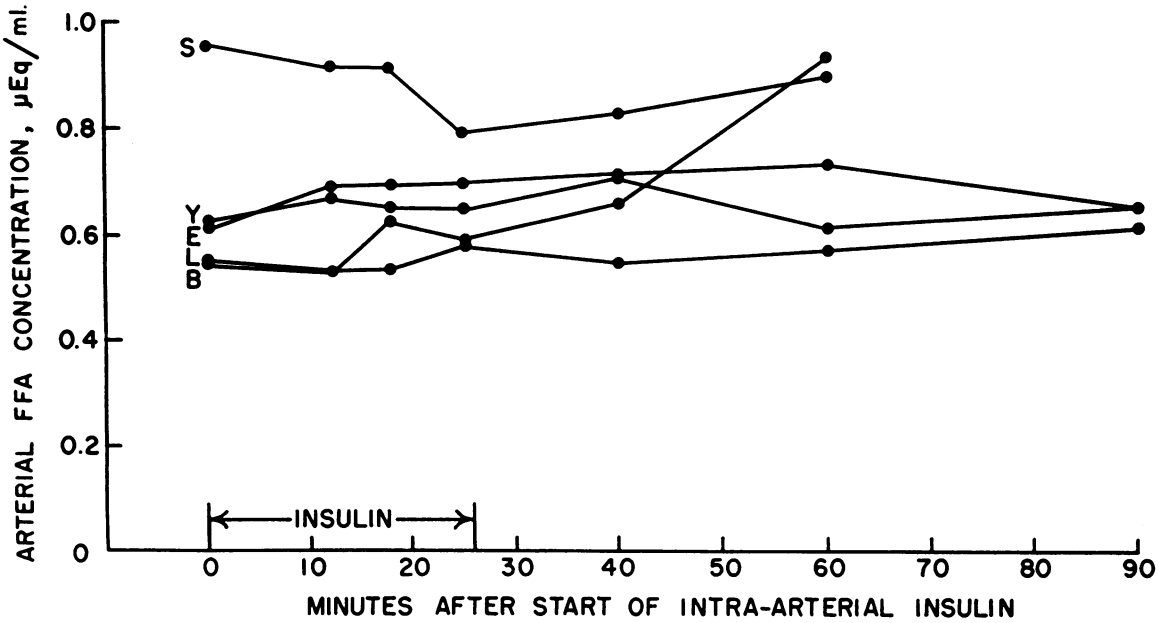

FIG. 1. LACK OF SYSTEMIC EFFECT OF INTRA-ARTERIAL INSULIN ON CONCENTRATION OF FFA IN ARTERIAL PLASMA. Each of the five subjects is identified by an initial.

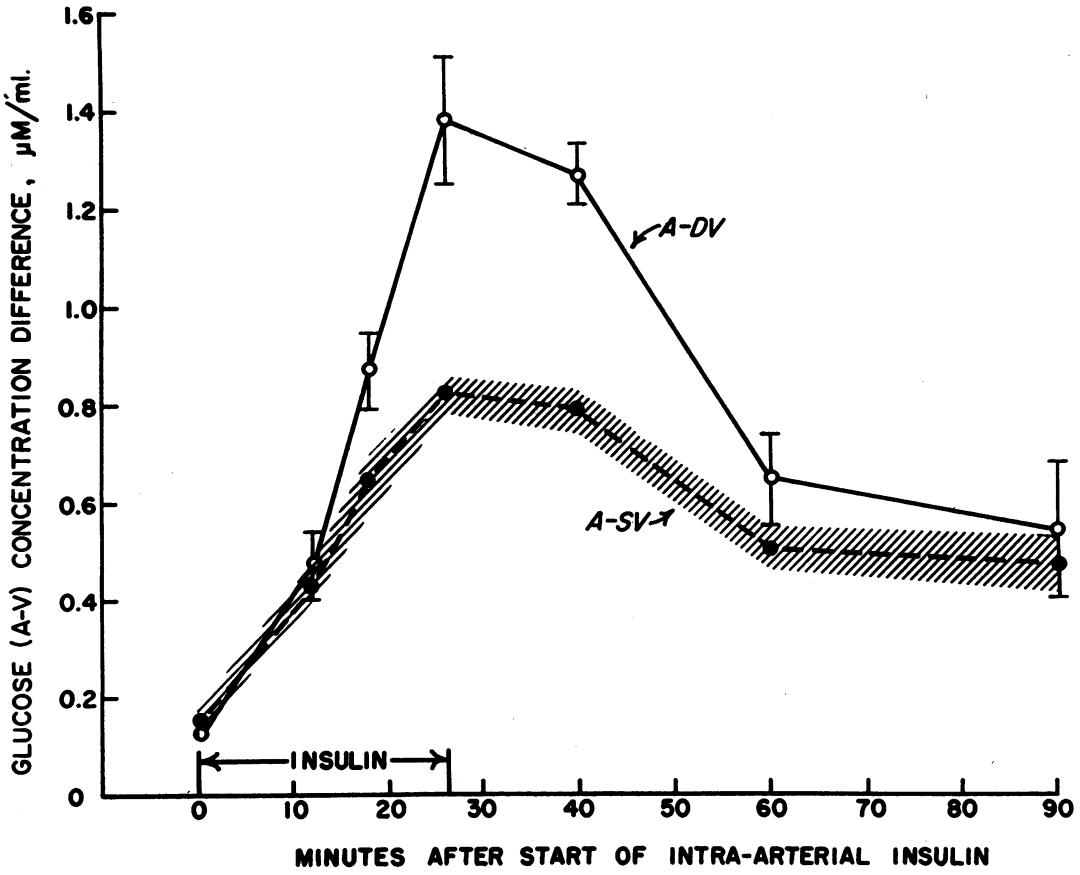

FIG. 2. GLUCOSE ARTERIO-DEEP VENOUS (A − DV) AND ARTERIO-SUPERFICIAL VENOUS (A − SV) CONCENTRATION DIFFERENCES ACROSS THE FOREARM IN RESPONSE TO INTRA-ARTERIAL INSULIN. Data at zero time are means of all samples taken before insulin administration, and are plotted as mean ± SE of mean.

ences were present in all five subjects, reflecting discharge of fatty acids from forearm adipose tissue into venous plasma. In each of the five subjects, negative A − SV FFA differences were reduced 12 minutes after start of the insulin infusion. Samples taken 18, 26, and 40 minutes from the beginning of insulin infusion showed complete inhibition of FFA release from the superficial bed. Thereafter, the effect decayed rapidly and negative A − SV FFA differences were restored by the 90-minute sample. Since blood flow through the superficial bed constitutes only about 10 per cent of forearm blood flow (1), the positive A − SV FFA difference uncovered at peak insulin effect is quantitatively small and probably represents uptake of FFA by nonadipose tissue drained by the superficial venous effluent, principally skin.

A − DV FFA differences (Figure 4). While values for basal A − DV FFA concentration differences showed wide variation, the mean value did not differ significantly from zero, in agreement with the larger body of evidence collected from this laboratory (2). After insulin administration, A − DV FFA differences became positive or more positive in all subjects. The magnitude of the response was related to the value of the basal A − DV difference: the largest increase occurred in the one subject, E, with a negative basal A − DV difference, indicating considerable admixture of DV by effluent from adipose tissue. Subjects exhibiting significantly positive basal A − DV differences in the neighborhood of 0.1 μmole per ml, and therefore, by inference, little contamination of DV by effluent from adipose tissue, showed only small increases with insulin. In all subjects, a positive A − DV difference of 0.07 μmole per ml or greater was uncovered under

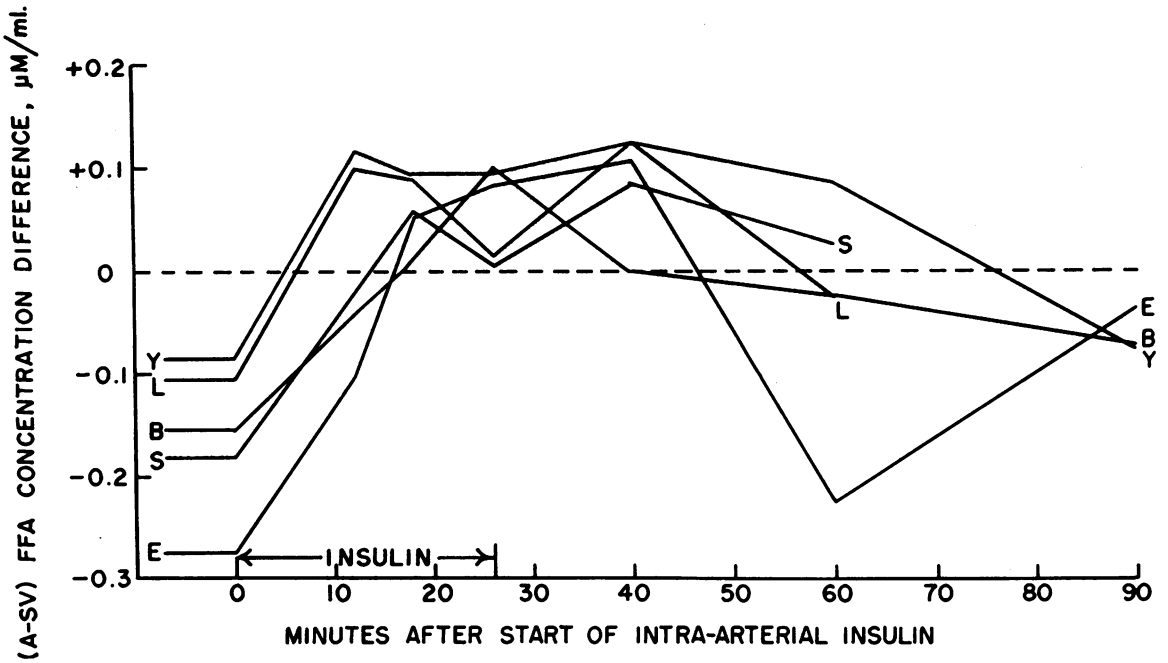

FIG. 3. ARTERIO-SUPERFICIAL VENOUS (A − SV) FFA CONCENTRATION DIFFERENCES ACROSS THE FOREARM IN RESPONSE TO INTRA-ARTERIAL INSULIN. Each of the five subjects is identified by an initial.

maximal insulin stimulation. The mean maximal A − DV difference achieved was 0.09 μmole per ml.

$\dot{Q}_{O_2}$, $\dot{Q}_{CO_2}$, and RQ. No significant changes in O_2 consumption, CO_2 production, or RQ were observed after insulin administration. This confirms earlier observations from this laboratory (14).

$G - L/O_2$. If glucose removed from arterial

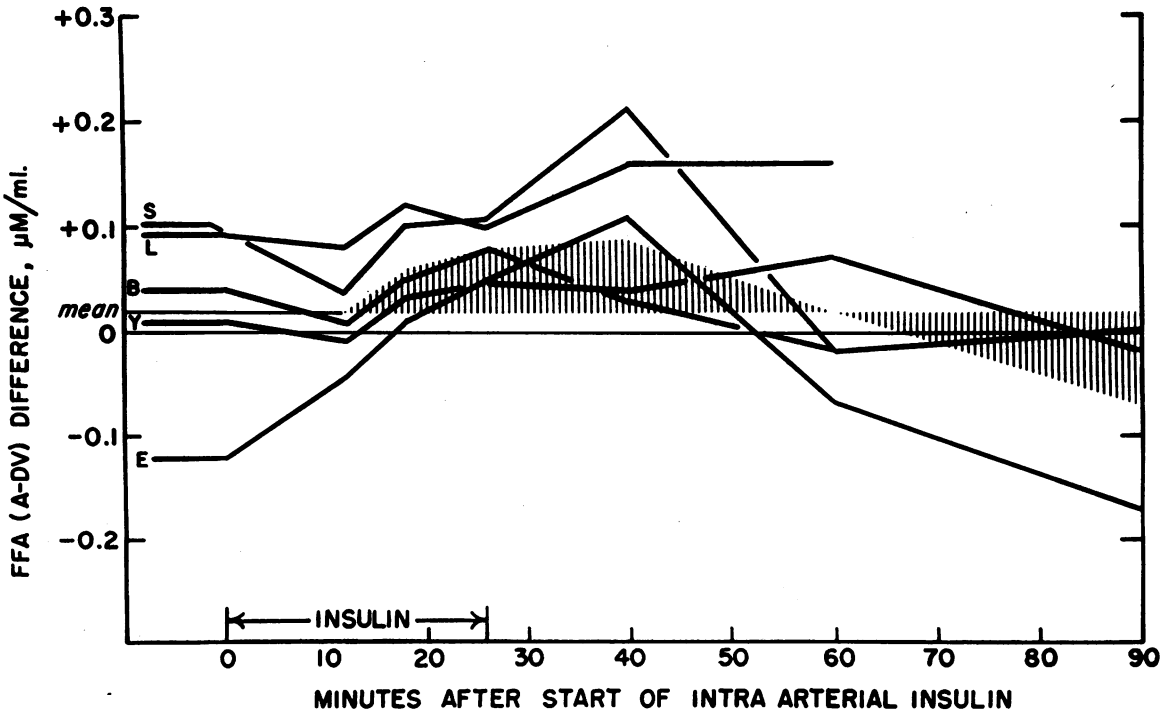

FIG. 4. ARTERIO-DEEP VENOUS (A − DV) FFA CONCENTRATION DIFFERENCES ACROSS THE FOREARM IN RESPONSE TO INTRA-ARTERIAL INSULIN. Shaded area gives the mean change in A − DV differences after insulin.

blood by forearm tissues but not accounted for by lactate production (G − L) were all oxidized, the fraction of observed O_2 consumption accounted for by glucose oxidation, G − L/O_2, was well above unity during insulin infusion; that is, more than enough glucose was abstracted by muscle to account for all its O_2 consumption. Evidence pointing to most of the incremental glucose being stored rather than oxidized under these conditions has been presented in detail elsewhere (14). In brief, failure of either $\dot{Q}_{O_2}$ or RQ to rise under insulin stimulation favors the notion that the glucose taken up by muscle is diverted toward glycogen synthesis. Calculation of G − L/O_2 ratio under these conditions is therefore meaningless.

FFA/O_2. Since 1 mole of FFA requires 25 moles of oxygen for complete oxidation, the fraction of O_2 uptake accounted for by oxidation of FFA abstracted from plasma, FFA/O_2, can be calculated from the following equation: $FFA/O_2 = [(A - DV)\ FFA \times 25 \times plasmacrit]/(A - DV)_{O_2}$. FFA/$O_2$ increased from an apparent mean basal ratio of 0.12 to a value of 0.42 or greater in each of the five subjects (Table II); that is, enough FFA was abstracted by muscle to account for about half its oxygen consumption under maximal insulin stimulation. Subjects L and S, with basal FFA/O_2 in the neighborhood of 0.5, suggesting only slight adipose tissue contamination of DV effluent, showed, as anticipated, little or no increase in FFA/O_2 with insulin; conversely, Subjects B, Y, and E, with either low or negative FFA/O_2, suggesting appreciable adipose tissue contamination of DV effluent, showed, again as anticipated, considerable increase in FFA/O_2 with insulin.

DISCUSSION

There have been a number of reports on effects of insulin on plasma FFA concentrations and on arteriovenous FFA concentration differences (4, 5, 16, 17). In most instances insulin was given intravenously, but even when administered intra-arterially, the dose was usually so large—usually 1 U or greater—that the effect was like that of an intravenous dose. The concentration of insulin in recirculating blood affecting total body response was generally as great as that affecting the forearm alone in the present study and, by provoking rapid changes in arterial concentration of glucose and FFA, made quantification of insulin action difficult.

Insulin-induced inhibition of FFA release has, however, been unequivocally shown across the femoral arterial-saphenous venous drainage bed (4, 5) and, insofar as A − SV concentration differences reflect metabolism of forearm adipose tissue, appears to be confirmed by our own data. At the same time, in each of the five subjects a positive A − DV FFA concentration difference of 0.07 μmole per ml or greater was unmasked, although insulin appears to be without direct influence on FFA uptake by peripheral tissues. The increase in A − DV FFA and FFA/O_2 varied inversely with the magnitude of basal values for these indices. This is consistent with the notion that insulin-induced positive A − DV FFA concentration difference results from inhibition of FFA release from forearm adipose tissue, since the largest insulin effect is seen in the subjects with the largest adipose tissue contamination of DV effluent and hence low or negative A − DV FFA and FFA/O_2. Insulin, therefore, merely unmasks FFA uptake by forearm muscle. In the light of these observations, the significance of an FFA/O_2 ratio of 0.5 under maximal insulin stimulation is that it implies that this may also be the amount of $\dot{Q}_{O_2}$ accounted for by FFA oxidation in the basal state.

Uptake of FFA by the forearm at peak response to insulin is 0.27 μmole FFA per minute per 100 ml forearm, or roughly 0.36 μmole FFA per minute per 100 g forearm skeletal muscle. FFA uptake by total skeletal muscle, which comprises 40 per cent of body weight, or approximately 25 kg, is thus approximately 0.1 mmole per minute. This figure corresponds very closely to the estimate of basal extrahepatic FFA oxidation given by Fritz in his comprehensive review of long-chain fatty acid oxidation (18).

Why FFA/O_2 ratio, even under maximal insulin stimulation, is closer to 0.5 than to the predicted 0.8 to 0.9 continues to pose a problem. One possibility arises from the suggestion that insulin may decrease the uptake of FFA by peripheral tissues (4). These observations, however, were made after glucose plus insulin administration in doses sufficient to cause a fall of 50 per cent or more in arterial FFA levels; under nonsteady-state conditions, A − V concentration differences

of zero do not necessarily imply absence of net movement of metabolites (19).

A second explanation for FFA/O_2 being in the neighborhood of 0.5 is the possibility that the missing fraction of 0.4 is provided by the diffusion of FFA from deep-lying adipose tissue into contiguous muscle through the interstitial fluid without traversing any vascular bed. These adipose tissue depots are probably replenished in the hours immediately after the ingestion of a mixed meal. Studies in the rat, based on distribution of injected, isotopically labeled chylomicron particles, support the notion that a significant percentage is deposited between or in fibers of skeletal muscle (9).

SUMMARY

The quantitative role of free fatty acids (FFA) in metabolism of forearm muscle in man was assessed by intra-arterial administration of insulin to five healthy young men: insulin-induced inhibition of FFA release from forearm adipose tissue unmasks an uptake of FFA by forearm muscle sufficient to account for about 50 per cent of its oxygen consumption. If insulin is without direct influence on the net uptake of FFA by muscle and adipose tissue, then this figure represents the quantitative role of plasma FFA in forearm metabolism under basal conditions as well.

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