Effect of Body Weight on Free Fatty Acid Release by Adipose Tissue *in vitro* [1]

Recognition of the metabolic importance of albumin bound free fatty acids (FFA) contributed extensively to our understanding of fat metabolism. It has been demonstrated that free fatty acids have a very fast turnover rate in the mammalian body and they can serve as energy sources for such vital organs as the heart, liver, etc.[2]. Adipose tissue is an active supplier of FFA both *in vivo*[3–5] and *in vitro*[6,7]. Hormonal influences on FFA release have been extensively studied, particularly *in vitro*, using the epididymal fat pad of the rat. Epinephrine, norepinephrine, ACTH, and growth hormone[8–11], stimulate, while insulin inhibits FFA release from adipose tissue[12–14].

The present paper reports an attempt to study release of FFA by adipose tissues of growing, grown and aged rats and to correlate the weight of animals with FFA release from their adipose tissue *in vitro*.

Materials and Methods. The male, albino rats of the Wistar strain used in these studies, were divided into three groups: Young, rapidly growing animals weighing less than 100 g, with an approximate age of 5–40 days (Group 1), mature rats weighing 180–300 g, with an approximate age of 80–120 days (Group 2), and old animals weighing 380–500 g, with an age-range of 5–16 months, the majority being 14–16 months old (Group 3). The animals were kept on an *ad libitum* diet. 18 h before the beginning of the experiments all food was removed from the cages. Adipose tissue samples were obtained under ether anesthesia, from the epididymal fat pad, the perirenal region, or the mesentery. The tissues were removed, rinsed with saline and with 1% solution of bovine serum albumin and were incubated in 5 ml incubation medium (5% bovine serum albumin in Krebs-Ringer phosphate buffer at pH 7.3–7.4) for 3 h according to the method of GORDON and CHERKES[6]. The incubation medium had a concentration of about 0.5 mEq/l of FFA. At the end of the experiment the tissues were blotted with filter paper and weighed. Only portions of the epididymal pads were used. Their weight ranged from 0.0039 to 0.2315 g in animals of Group 1 from 0.0075 to 0.6263 g in Group 2, and from 0.008 to 0.2727 g in Group 3. In experiments designed to study the effect of epinephrine both the treated and control tissues were taken from the same animals. FFA was determined by DOLE's method[15], triglycerides by the method of VAN HANDEL and ZILVERSMIT[16].

Results. Table I compares the 3 groups of rats with regard to epididymal FFA release in absence or presence of epinephrine and to mesenteric and perirenal adipose tissue release. Release of epididymal adipose tissue was significantly higher ($p < 0.001$) in Group 1 than in either Group 2 or 3. No significant difference existed between Group 2 and 3. Addition of epinephrine (0.005 mg) to the incubation medium increased the release of FFA from the epididymal adipose tissue in all 3 groups. The release was significantly higher ($p < 0.001$) in Group 1 than in Group 2 or 3. However, Group 3 showed significantly ($p < 0001$) higher relase than Group 2.

Mesenteric adipose tissue taken from Group 1 rats released significantly ($p < 0.001$) more FFA than either Group 2 or 3. No significant difference was found between Group 2 and 3.

Study of FFA release by perirenal adipose tissue was also carried out, but due to the scarcity of this tissue in very young animals, only tissues from Group 2 could be compared with those from Group 3. No significant difference was found between these 2 groups.

The FFA content of the tissues was not significantly different in the various groups either before, or after incubation (Table 2). The triglyceride content was lowest in fat pads of animals in Group 1, considerably higher

Tab. I. Release of FFA by adipose tissue in rats of different weights

	Group 1	Group 2	Group 3
Weight in g	< 100	180–300	380–500
Age, days	15–40	80–120	150–480
FFA release[a] (μEq/g/h)	24.76 ± 3.63[b] (20)[c]	4.77 ± 0.57 (12)	6.5 ± 0.91 (18)
FFA release after epinephrine[a] (μEq/g/h)	45.59 ± 7.68 (14)	10.54 ± 0.98 (6)	20.81 ± 2.38 (17)
FFA release by mesenteric adipose tissue (μEq/g/h)	32.73 ± 10.45 (12)	4.3 ± 0.82 (11)	10.82 ± 6.16 (6)
FFA release by perirenal adipose tissue (μEq/g/h)	—	5.85 ± 0.65 (12)	5.56 ± 2.21 (5)

[a] Epididymal fat pad. [b] Standard error. [c] No. of animals.

Tab. II. Tissue FFA, triglyceride and nitrogen content in rats of different weights

	Group 1	Group 2	Group 3
Weight in g	< 100	180–300	380–500
Age, days	15–40	80–120	150–480
Tissue FFA before incubation[a] (μEq/g)	20.14 ± 2.24[b] (17)[c]	19.48 ± 0.97 (12)	20.22 ± 2.22 (21)
Tissue FFA after incubation[a] (μEq/g)	29.9 ± 4.51 (11)	—	31.42 ± 6.82 (13)
Tissue triglyceride before incubation[a] (mg/g)	184.65 ± 31.6 (20)	583.0 ± 29.46 (12)	845.51 ± 18.13 (20)
Tissue nitrogen before incubation[a] (mg/g)	11.18 ± 1.35 (11)	5.10 ± 0.54 (12)	3.67 ± 0.51 (14)

[a] Epididymal fat pad. [b] Standard error. [c] No. of animals.

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in Group 2, and highest in Group 3. These differences between the groups were highly significant ($p < 0.001$). The nitrogen content on the other hand, was significantly higher ($p < 0.001$) in Group 1 than in Group 2 or Group 3. The difference between the latter 2 groups was not significant.

Discussion. The youngest, most rapidly growing rats released the most FFA per unit weight of the tissue *in vitro*. The nitrogen content per unit weight was also the highest in this group. The difference between the groups was much less striking if release of FFA was expressed per nitrogen content of the tissue. Tissue triglyceride content was highest in the oldest group and lowest in the youngest group. These differences became much more marked when expressed per nitrogen content of the tissue. Since FFA release by adipose tissue is under the influence of tissue lipases, it could be assumed that either concentration and/or activity of these lipases was greater in the younger animals than in the older ones.

It is not clear at the present time, what bearing these findings have on *in vivo* conditions. It might be speculated that there is a relationship between more extensive accumulation of adipose tissue with advancing age and the decreased release of FFA from the adipose tissue.

It might also be suggested that the young rapidly growing rats have an abundant supply of growth hormone which could be responsible for the increased release and decreased accumulation of FFA, but this cannot be determined with presently available information. Finally, it has been observed[17] that tissues from young animals are more responsive to the metabolic effects of insulin than tissues removed from older rats. The connection, if any between this finding and the presently reported observations has not been clarified.

Zusammenfassung. Die Absonderung freier Fettsäuren aus dem Fettgewebe wurde bei verschieden alten Ratten (1. Gruppe unter 100 g, 2. Gruppe 180–300 g und 3. Gruppe 380–500 g) *in vitro* ermittelt. Für das Fettgewebe der Nebenhoden ergab sich in der 1. Gruppe die stärkste Absonderung freier Fettsäuren und deren wirksamste Förderung durch Epinephrin. Hingegen war der Gehalt an 3-Glyceriden in der 1. Gruppe am niedrigsten, in der 3. Gruppe am höchsten.

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On the *in vitro* Corticotropin Releasing Factor (CRF) Activity of the Heptapeptide: Methionyl-Glutaminyl-Histidyl-Phenylalanyl-Arginyl-Tryptophyl-Glycine

In a preceeding publication[1], we have mentioned the *in vitro* CRF activity measured by the method of SAFFRAN et al.[2] of the heptapeptide H-Met-Glu(NH_2)-His-Phe-Arg-Try-Gly-OH synthesized by KAPPELER and SCHWYZER[3,4]. This heptapeptide was already known for its melanocyte stimulating activity (MSH activity); it represents a sequence of seven amino acids common to the hormones of the corticotropins (ACTH) and MSH group.

Later on, the heptapeptide: H-Met-Glu-His-Phe-Arg-Try-Gly-OH which differs from the preceeding one only by the absence of an amide group, was synthesized by LI et al. and found to have both melanocyte stimulating and corticotropin releasing activities (MSH and CRF activities)[5].

In the present note, we describe a series of *in vitro* assays of the heptapeptide H-Met-Glu(NH_2)-His-Phe-Arg-Try-Gly-OH performed in our laboratory between December 1959 and October 1961. A large number of determinations made during this period gave consistant results, some of the most significant of which have been collected in the Table.

The experimental details are those described by SAFFRAN et al.[2]. The CRF activity may be expressed either: (1) as the minimal dose of active material which is able to provoke a statistically significant increase of the secretion of ACTH by the anterior pituitary of rats, versus non-stimulated anterior pituitaries; (2) as the dose of active material able to produce a response equal to the response given by a constant standard preparation.

At the present time, both methods of expression are used; in the Table we have used uniformly the second mode of expression so that recent results may be compared to the former ones calculated according to the second mode. The rats utilized for the assays were male animals of the Wistar U.S.A. strain, weighing 150 g.

Different doses of the heptapeptide were tested versus a constant uniform dose of 4 µg of CRF 91[6]; this dose of 4 µg of CRF 91 was considered as a provisionnal unit of CRF activity. The activity of any unknown material was expressed by the ratio between the amount of ACTH released by this material versus the amount of ACTH released by 4 µg of CRF 91. Generally speaking this ratio varies between a rather narrow range and several hundred assays performed so far have shown that: a ratio 0.5 means no stimulation; a ratio between 0.5 and 1.0 means weak stimulation; a ratio between 1.0 and 1.5 means medium stimulation; a ratio between 1.5 and 2.0 means high stimulation.

The results of these determinations were evaluated by standard statistical tests[8], the limits of confidence were calculated for the ratios as well as the 'departure from parallelism'. The assays reported in the Table do not show any departure from parallelism exceeding the critical values. The data of the Table show that the heptapeptide is able to release an amount of ACTH greater than the amount released by 4 µg of CRF 91 at the doses of 4, 2, 1,

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