Release of free fatty acids from adipose tissue obtained from newborn infants

MILAN NOVÁK, VÁCLAV MELICHAR, PETR HAHN, and OTAKAR KOLDOVSKÝ

Institute for the Care of Mother and Child and Institute of Physiology, Czechoslovak Academy of Sciences, Prague, Czechoslovakia

SUMMARY The role played by mobilization of free fatty acids (FFA) from adipose tissue in producing the typically high serum FFA levels of human infants has been studied. FFA concentrations in the serum and subcutaneous adipose tissue from the gluteal region were determined during postnatal development. A maximum level was reached within 24 hr after birth, after which there was a gradual fall. In serum the FFA level at the end of 12 months was still higher than that in adults, while the FFA level in adipose tissue was lower at 3 months than in adults.

Incubation of small pieces of adipose tissue in Krebs-Ringer phosphate buffer containing 4% albumin led to release of FFA into the medium. This release could be suppressed by the addition of glucose (200 mg/100 ml) for tissue from all age groups except the youngest (0–15 hr after birth).

KEY WORDS free fatty acids · adipose tissue · infants · incubation · needle aspiration · metabolism

In the early postnatal period of most mammals (e.g., rat, sheep) fat is the main source of energy (1, 2), and this also holds for human infants (3, 4). This fat is derived mainly from the diet (milk) and it is not clear to what extent endogenous fat can be mobilized during the suckling period. In infant rats it seems that during starvation endogenous fat reserves are utilized to a greater extent than in adults, since it has been shown that such animals use up much more of their fat stores and less of their body proteins than adult animals (5). Very little, however, is known about fat mobilization mechanisms in man. In vitro studies of adipose tissue from adult men have been performed only rarely, generally using tissue obtained during surgery or after death (6–8). Hirsch et al. (9) have developed a simple method for obtaining samples of human adipose tissue suitable for analyzing its fatty acid composition. On the other hand, adult rat adipose tissue has been extensively utilized for in vitro studies (10) and many of the metabolic and endocrine factors regulating release of free fatty acids (FFA) have been elucidated.

It has been shown in previous work (4, 11) that the level of FFA in the blood rises rapidly after birth and remains elevated throughout the suckling period in human infants. Similar results have been obtained by Kaye and Kumaga (12) and by Van Duyne et al. (13) in sheep.

In the present paper the in vitro release of FFA by adipose tissue obtained from human infants has been studied in order to evaluate to what extent FFA mobilization from adipose tissue may be responsible for the high FFA levels found in newborn infants. Levels of FFA in the serum and adipose tissue during development have also been determined.

METHODS

FFA Determination in Adipose Tissue

Adipose tissue from the gluteal region was obtained by the method of Novák and Melichar (14). Basically the method consists of taking a sample of intact adipose tissue using a special needle with a trocar.

Healthy adults and infants were used. The infants were deprived of food for 6–8 hr, adults (who were on a normal diet) for 12 hr. Newborn infants (younger than 24 hr) had never received food before samples were taken; 3-to 6-month old infants were fed breast milk and artificial milk mixtures. The sample of gluteal adipose tissue, weighing 5–15 mg, was expressed from the needle into Krebs-Ringer phosphate buffer, rinsed, carefully dried on filter paper, and then placed into a preweighed glass-stoppered small test tube and weighed. Then 0.6

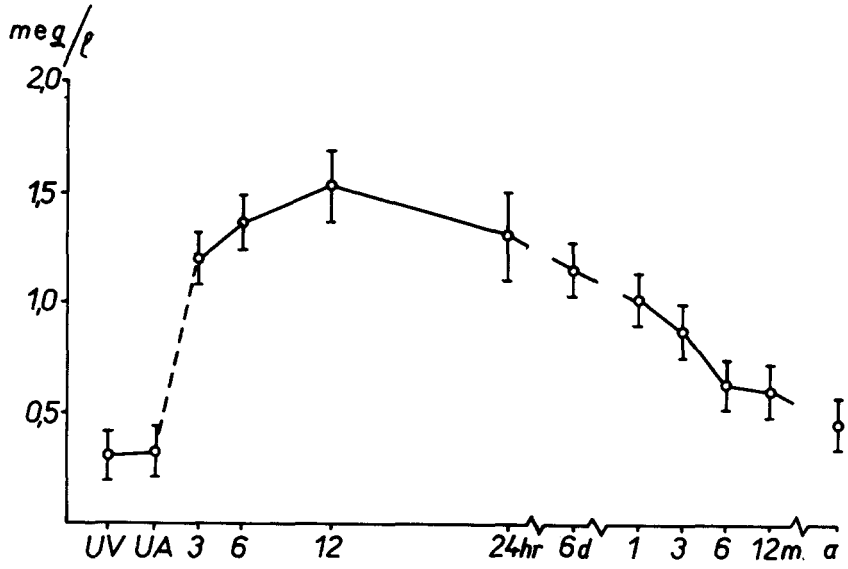

FIG. 1. Changes in FFA level of serum during development. Abscissa: age in hours (hr), days (d), months (m), and adults (a). *UV* = umbilical vein, *UA* = umbilical artery. Ordinate: meq/liter FFA. Vertical lines indicate ± SE. For each age group 15 infants were used, except for the 3- and 12-month groups (7 infants each).

ml of the extraction mixture of Dole (15) was added. The tissue was homogenized and the homogenate left to stand for 3 hr in a refrigerator, by which time extraction was complete, while evaporative loss of solvents was minimized. FFA were then determined according to Dole's method as modified by Trout et al. (17) and for ultramicro determination by Novák (16). The ultramicro determination is based on the use of a specially constructed burette and of constriction pipettes of precisely known volume. Special glass for ultramicro analysis was used.

Release of FFA from Human Adipose Tissue In Vitro

The piece of tissue (7–20 mg) was placed on a glass slide and cut horizontally by 3 cuts (using a razor blade), so that the cylindrical piece was of fairly constant surface. It was then transferred to a small glass-stoppered test tube and weighed. The test tube contained Krebs-Ringer phosphate buffer (pH 7.4) without Ca^{++} and with 4% bovine albumin (bovine plasma albumin fraction V, Armour Pharmaceutical Co. Ltd., Eastbourne, England). For every 10 mg of tissue, 0.2 ml of buffer was added using an Agla micropipette. The time between taking the sample and incubating it was never more than 1 min. Samples were incubated for 90 min at 37° and 120 shakes/min. FFA were determined at the end of the incubation period using the ultramicro method of Novák (16).

Samples of tissue taken from the same subject released FFA at the same rate. A test tube containing the medium only was run as a control and the final level of FFA was subtracted from all other values. Capillary blood was collected from the heel into a glass capillary in the usual way at birth (see times in Fig. 1) and in the morning before feeding in all other age groups.

RESULTS

FFA Level in Serum and Adipose Tissue during the First Year of Life

Figure 1 shows that the highest serum FFA level was found on the first day after birth. Then there is a gradual fall, but at 12 months the level is still higher than in adults.

It is evident from Fig. 2 that the FFA content of adipose tissue runs a course parallel to that found in the serum. The highest levels were again found on the first day of life. At 3 months, however, the level is lower than in adults.

In Vitro Release of FFA from Adipose Tissue

Figure 3 shows that there is a nonlinear release rate, most of the FFA being released between 30 and 60 min. Figure 4 demonstrates that there are only small age differences in the rate of release of FFA from adipose tissue. The effect of adding glucose on FFA release is shown in Figs. 4 and 5. It was found that immediately after birth until about 15 hr later, glucose addition to the medium did not suppress the release of FFA, while at all later ages the normal response was observed, i.e., suppression of FFA release. Figure 5 shows the time course of FFA

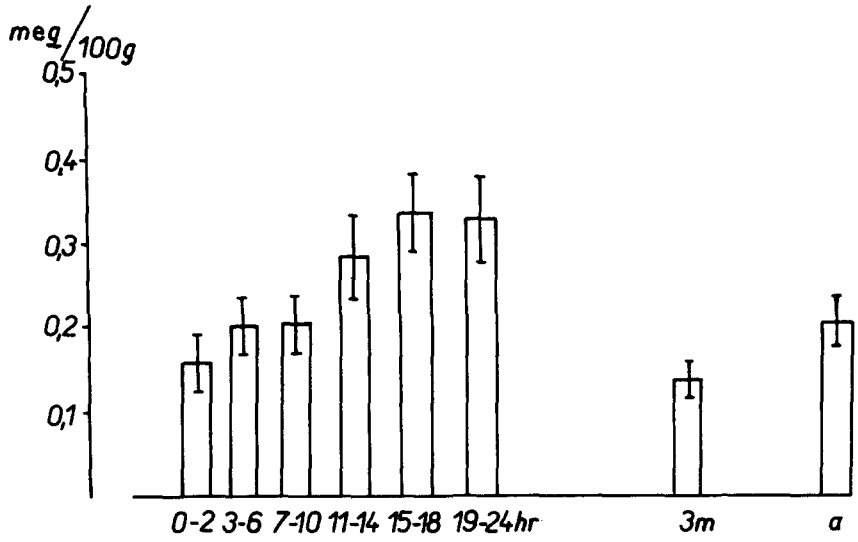

FIG. 2. Changes in the FFA content of adipose tissue during development. Abscissa: age in hours (hr), months (m); adults (a). Vertical lines = ±SE. Each column is the mean of 6–8 determinations from 6–8 infants.

release, in the presence of glucose, from adipose tissue of newborn infants younger and older than 18 hr and demonstrates that the effect of glucose becomes more pronounced as the incubation period is prolonged.

DISCUSSION

It appears from this work that the early postnatal rise in FFA content of the serum is due to release from adipose tissue, since at that time no food has as yet been consumed. It seems highly improbable that the postnatal rise in serum FFA content is due to decreased utilization of these acids since (*a*) administration of glucose suppresses this rise (4) and (*b*) there is a pronounced rise in ketone body levels in serum (Melichar and Drahota, unpublished work). On the other hand, it may be postulated that later in life the high FFA level in the serum is not due to release from adipose tissue but to supply of FFA from food. Thus at 3 months the serum level is higher than in adults while the content of FFA in adipose tissue is lower. It is, of course, also possible that the total fat content of a 3-month old infant is so high that less release per unit adipose tissue weight is required to raise the serum level.

The fact that addition of glucose to the medium does not suppress FFA release from adipose tissue immediately after birth is surprising in view of our previous finding that administration of glucose to newborn infants always

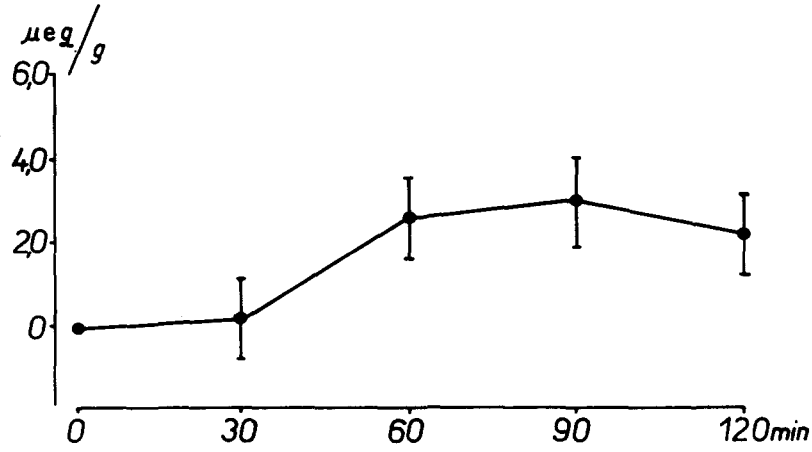

FIG. 3. Release of FFA (into Krebs-Ringer phosphate buffer containing 4% albumin) by adipose tissue from newborn infants. Abscissa: time in min. Ordinate: μeq FFA per g of tissue. Vertical lines = ±SE. Each point is the mean of results from 6 infants.

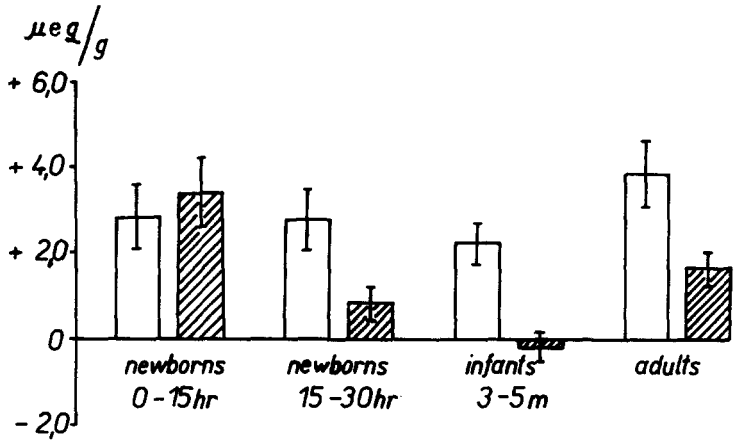

FIG. 4. The effect of adding glucose (200 mg/100 ml) on release of FFA from adipose tissue in vitro. Abscissa: age in hours (hr), months (m). White, without glucose; shaded, with glucose added. Vertical lines = ±SE. Significance of differences as determined by paired "*t*" test:

Effect of glucose: $P < 0.01$ for 15–30 hr, $P < 0.001$ for 3 months, $P < 0.002$ for adults

Effect of age (percentage decrease of FFA release on glucose addition): $P < 0.02$ for adults vs. 3 months. The columns from left to right are the mean of results from 15, 8, 8, 8, 7, 6, 6, and 6 infants, respectively.

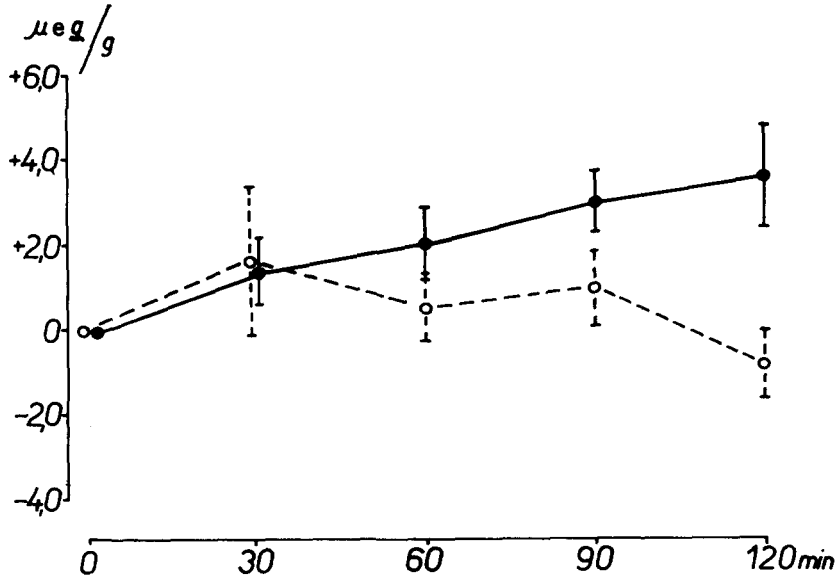

FIG. 5. FFA release from adipose tissue in vitro in the presence of glucose. Abscissa: time of incubation in min (incubation). Ordinate: μeq/g. Dashed line, infants older than 18 hr; full line, infants younger than 8 hr. Vertical lines indicate ± SE. Each curve is the mean of results from 6 infants.

decreases the level of FFA in the serum (4). It must be borne in mind that in vitro conditions may not simulate in vivo conditions well. Further work will also be required to explain why following the intravenous administration of glucose to newborn infants there was a rise in the level of esterified fatty acids in the serum (4). It is just possible that esterification of FFA at that period of life occurs outside the adipose tissue pool.

In the work of Hamosh et al. (8) the release of FFA was only 1μeq/g in 2 hr as compared to about 2 μeq/g per hr in our experiments. This difference might be explained by (*a*) the use of tissue obtained from anes-

thetized patients, and (*b*) the much larger pieces of adipose tissue used for incubation (250 mg).

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