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Adipose tissue glycogen
ATP neonate
fetus

The Metabolism of Subcutaneous Adipose Tissue in the Immediate Postnatal Period of Human Neonates. III. Role of Fetal Glycogen in Lipolysis and Fatty Acid Esterification in the First Hours of Life

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Extract

Total glycogen phosphorylase activity in neonatal adipose tissue is significantly increased immediately after birth (0–6 hr) and rapidly decreases to a relatively constant level. Fluoride-stimulated adenyl cyclase activity in homogenates of adipose tissue from neonates is directly correlated with the length of time from the onset of labor ($y = 0.665 + 7.88x$; $P < 0.02$). No significant differences are found between infants 1–10 hr of age and 2–4 days of age. The ATP content decreases rapidly in the first hours of life and is inversely correlated with the length of time since the onset of labor ($\log y = -0.657 - 0.0164x$; $P < 0.01$). The ATP content starts to increase approximately 24 hr after birth. Changes in ATP content are directly correlated with glycogen content and glycerol release from neonatal adipose tissue.

Speculation

The intensity of lipolysis in neonatal adipose tissue depends on various factors, one of the most important being an adequate energy supply. The increased glycogen content in adipose tissue immediately after birth and the rapid rate of utilization of glycogen-derived glucose appear to be major factors involved in the production of ATP required for glycogenolysis, lipolysis, and fatty acid activation during the early adaptation of the neonate to extrauterine life.

Introduction

The early adaptation of the fetus to extrauterine life depends mainly on conversion of the potential energy of substrate stores into utilizable form. Before the neonate is capable of covering his energy requirements by ingestion of sufficient milk, he is dependent on his own endogenous energy sources. This time may be charac-

terized as a period of maximal mobilization of energy depots. Two basic substances are deposited in the fetus and utilized by the neonate as sources of energy: carbohydrates, mostly as glycogen, and lipids, predominately as triglycerides.

Elevated glycogen deposits have been found in the subcutaneous adipose tissue of human neonates immediately after birth [25]. As in other tissues [35], the glycogen level in the subcutaneous adipose tissue falls rapidly in the first hours after delivery [25].

Increased fat mobilization also begins in the first hours of life. It may be documented by a rapid rate of increase of free glycerol [20] and free fatty acids (FFA) in the blood [24, 37] and by changes in glycerol release from subcutaneous adipose tissue *in vitro* [22, 25]. Most of the glycerol formed in the period of accelerated lipolysis leaves the adipose cell while substantial amounts of fatty acids undergo immediate re-esterification. According to *in vitro* studies of subcutaneous adipose tissue in human neonates [22, 25], lipolysis increases rapidly after birth, reaches a maximum within a short period and then gradually decreases. Glycogenolysis, on the other hand, seems to be initiated before delivery during the process of labor, and the breakdown of glycogen can contribute both the α-glycerophosphate and the ATP required for fatty acid re-esterification during this period. However, fatty acids later accumulate in the adipose tissue and increasingly leave the fat cell, which may be related to the lack in older neonates of available carbohydrates. Triglyceride hydrolysis is catalyzed by a hormone-sensitive lipase which is most probably the rate-limiting enzyme in triglyceride breakdown, and the adenyl cyclase system is involved in the activation of this enzyme [6, 7, 31, 39]. The substrate for cyclic adenosine 3′,5′-monophosphate (cyclic AMP) formation is ATP, and this is consumed in the activation of free fatty acids for re-esterification. It may also be required in the activation of hormone sensitive lipase, analogously to glycogen phosphorylase [39].

In the present studies, age-related changes in total and active glycogen phosphorylase, fluoride-stimulated adenyl cyclase, and ATP content were measured *in vitro* in subcutaneous adipose tissue from normal newborn human infants. The relation between these variables of adipose tissue function are related to previous findings in regard to changes in glycogen content and utilization of carbohydrates in the subcutaneous adipose tissue, and also to glycerol release as an indicator of triglyceride breakdown.

Materials and Methods

Subcutaneous adipose tissue was obtained from the gluteal region of normal newborn infants of different ages, between 1 hr and 10 days, using a needle biopsy technique. Criteria for the selection of normal neonates were the same as previously outlined [25].

Samples, about 10–40 mg, were either put immediately in a small vial on Dry Ice and kept frozen until analysis or dispersed in $HClO_4$ and cooled in an acetone-Dry Ice mixture when ATP was measured. The procedures from the time the sample is cut off from its circulation to the time of freezing or fixation require approximately 15 sec.

Glycogen Phosphorylase Activity

Five age groups were used in this part of the study: 0–6, 6–12, 12–48, 48–96, and 120–168 hr with six to nine infants included in each group. Total and active glycogen phosphorylase were estimated by the method of Greenberg and Glick [9], used by these authors in rat adrenal. As modified for adipose tissue, the reaction was found to be linear with time and amount of tissue. Thin sections of the fresh frozen adipose tissue were cut with a scalpel blade and incubated in 20 μl reaction mixture which consisted of exogenous glycogen [41] 4 mg/ml, in 0.05 M phosphate buffer, pH 6.1, and contained 0.1 M NaF. If the total glycogen phosphorylase was being determined, 0.57 mg/ml of 5′-adenosine monophosphate [42] was added to the medium. After 60 min incubation at 37°, the reaction was stopped by adding 100 μl absolute ethanol. The samples were cooled 30 min at 4° and the precipitated glycogen removed by 10 min centrifugation at 4°. This centrifugation also removed the tissue residues. An aliquot (60 μl) of the ethanolic extract, which contained the reaction product, glucose 1-phosphate, was evaporated to dryness under reduced pressure and hydrolyzed with 20 μl 0.9 N HCl at 100° for 7 min. With the limited amounts of tissue obtainable by needle biopsy, the estimation of glucose 1-phosphate-derived glucose was found to be more practical than determination of changes in inorganic phosphate. After neutralization with NaOH, the glucose content was measured using the glucose oxidase method [43]. For each series of estimations, a parallel blank containing all of the reagents except the tissue sample was carried through the procedures. The remainder of the material in the tube contains the adipose tissue protein and was used to evaluate sample size by measuring its protein con-

tent by the Lowry method [16]. Results are expressed as micromoles of glucose 1-phosphate generated per gram of protein per 60 minutes.

Fluoride-stimulated Adenyl Cyclase Activity

In this part of the study a group of eight neonates 0–10 hr of age were compared with a group of eight infants 2–5 days of age.

Adenyl cyclase activity was assayed according to the method of Krishna *et al.* [15]. In principle, enzyme activity is measured in terms of the rate of formation of labeled cyclic AMP from ^{14}C-labeled ATP, in an incubation medium which consisted of 0.14 M Tris-HCl buffer, pH 7.3, 0.0033 M $MgSO_4$, 0.01 M NaF, and 0.01 M theophylline. After homogenization of the sample in 0.3 ml of this buffer with a sonic vibrator at 0°, the sample was centrifuged in a refrigerated centrifuge at 3,500 rpm for 15 min. A 200-μl aliquot of the fat-free sonic supernatant was used for incubation (30 min) with 400 μl medium in vials containing 8-^{14}C-labeled ATP (1 μmol, specific activity 1.01 mCi/mM) [44] at 30°. The reactions were stopped by adding 100 μl carrier cyclic AMP solution and heating in boiling water for 2 min.

The contents of the reaction mixture were transferred to a column which contained 2 ml 50% v/v suspension of Dowex 50-H^+ [45] for ion exchange chromatography. The part of the effluent containing the cyclic AMP was treated with $Ba(OH)_2$-$ZnSO_4$, which precipitates all other nucleotides, and also inorganic phosphates. Before use in the study, the method of separation of cyclic AMP was evaluated with ATP (both labeled and cold), ADP, 5′-AMP, and cyclic AMP and found to be adequately specific and quantitative. ^{14}C-Labeled cyclic AMP was counted using a Nuclear Mark II scintillation counter in Bray's scintillation fluid. The homogenate (10 μl) was also used for protein determination by the method of Lowry *et al.* [16]. Results were related to the total protein of the homogenate (millimicromoles of cyclic AMP generated per gram of protein per minute).

Adenosine 5′-Triphosphate Content

Samples were obtained from 55 neonates who were 1–200 hr of age. Before the sampling, 200 μl 0.6 N perchloric acid was measured into a small, narrow test tube equipped with a glass stirring rod, a stopper, and a plastic stand to support the tube in an upright position; all were weighed together on an analytic balance. Immediately after being obtained, the sample was placed in the ice-cold 0.6 N perchloric acid. A tissue sample of not more than 10–20 mg was used. The tissue was minced in the perchloric acid using the stylet of the biopsy needle and then frozen immediately in a Dry Ice-acetone mixture. In this form the sample was transported to the laboratory where sample, tube, glass rod, stopper, and support stand were weighed together at room temperature to determine the sample size difference. The homogenization and extraction of the sample was accomplished with the glass rod during the thawing of the perchloric acid containing the sample fragments.

After centrifugation, an aliquot of 100 μl was removed from below the upper fat layer with a Hamilton syringe. The ATP content was estimated according to the method of Adam [1] using a Boehringer-Mannheim test combination [46]. The decrease in NADH was registered at 340 nm with a Cary 11 spectrophotometer using a 0–0.1 OD slide wire. Calibration was with pure ATP [46]. Results are expressed as milligrams of ATP per gram wet weight.

Results

Total glycogen phosphorylase activity (micromoles of glucose 1-phosphate generated per gram of protein per hour) measured in thin sections of adipose tissue decreases rapidly after delivery to a nearly constant level (Fig. 1). Mean glycogen phosphorylase activity was

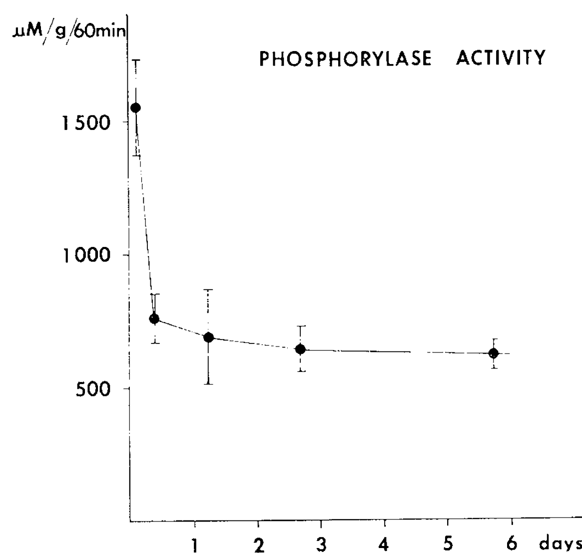

Fig. 1. Aged-dependent changes in total glycogen phosphorylase (micromoles of glucose 1-phosphate generated per gram of protein per hour) in thin sections of adipose tissue from normal neonates in the 1st week of life.

Table I. Age-dependent changes in total and active glycogen phosphorylase activity in subcutaneous adipose tissue from normal full term human neonates[1]

Age		Total[2]	Active[3]	Active, %[4]
hr	days			
2.5–3.5		1,110	665	60
		1,110	555	50
		2,100	1,000	48
		700	556	80
		2,440	890	37
		1,440	1,100	77
12–24		532	444	83
		775	445	58
		450	332	74
		520	222	43
	2–7	456	400	80
		632	456	50
		445	310	70
		1,110	334	30
		456	400	80

[1] Total and active phosphorylase were significantly greater between 2.5 and 3.5 hr of age than between 12 and 24 hr ($P < 0.05$ by Wilcoxon test) and 2 and 7 days ($P < 0.01$).
[2] Glucose 1-phosphate micromoles generated per gram of protein per hour with 5′-AMP in the medium.
[3] Glucose 1-phosphate micromoles generated per gram of protein per hour without 5′ AMP in the medium.
[4] Ratio of active/total × 100.

1,556 ± 196 (mean ± SE) in infants 0–6 hr of age ($n = 9$); 758 ± 93, 6–12 hr of age ($n = 7$); 686 ± 177, 12–48 hr of age ($n = 7$); 639 ± 89, 48–96 hr of age ($n = 5$); and 625 ± 63, 120–168 hr of age ($n = 6$). Glycogen phosphorylase activity was significantly increased between 0–6 hr of age in comparison with the 6–12-hr-old group ($P < 0.01$ by Student's t test) and with all older groups ($P < 0.01$ or 0.001). In a second study in which both total and active phosphorylase were measured, active glycogen phosphorylase (phosphorylase *a*) was also increased ($P < 0.05$, Wilcoxon test) in the youngest age group (Table I). The percentage of phosphorylase in the active form amounted to approximately 60% of the total phosphorylase and showed no trend with age.

In infants less than 1 day old, no relation was found between glycogen phosphorylase in the adipose tissue and the length of time since the onset of labor ($n = 22$). In the paired analyses for glycogen and glycogen phosphorylase, there was no significant correlation between the two variables in the 1st day of life ($n = 14$).

Mean fluoride-stimulated adenyl cyclase activity (millimicromoles of cyclic AMP generated per gram of protein per minute) in crude homogenates of adipose tissue was 15.7 ± 1.1 (mean ± SE) ($n = 8$) in the first 10 hr of life and was significantly correlated with the length of time since the onset of labor, $y = -665 + 7.88x$, $r = 0.851$, $P < 0.02$ (Fig. 2). In the first 10 hr of life there was no significant correlation between adenyl cyclase and age, unless the length of time since the onset of labor was taken into account. No significant difference was found between the group 1–10 hr of age and 2–4 days of age, whose mean fluoride-stimulated adenyl cyclase was observed to be 15.1 ± 1.7 ($n = 8$).

The ATP content (milligrams per gram wet weight) decreased rapidly in the first hours of life, reaching a minimum at the end of the 1st day, and then slowly increased (Fig. 3). The ATP content was 0.185 ± 0.011 (mean ± SE) between 3.5 and 6.5 hr of age ($n = 18$). It was significantly decreased ($P < 0.001$) between 10 and 33 hr of age, 0.0785 ± 0.0094 ($n = 16$), in comparison with the youngest group, and was significantly in-

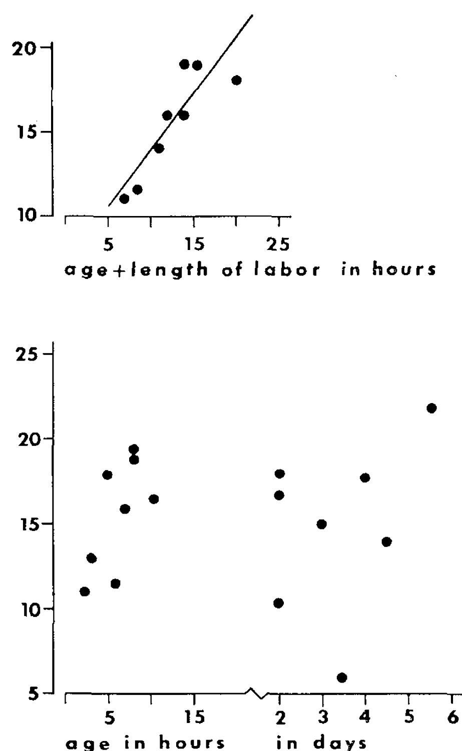

Fig. 2. Age-dependent changes in the activity of fluoride-stimulated adenyl cyclase (millimicromoles of cyclic adenosine 3′,5′-monophosphate generated per gram of protein per minute) in homogenates of subcutaneous adipose tissue from normal newborn infants in the 1st week of life (*below*) and the effect of the length of time since the onset of labor on fluoride-stimulated adenyl cyclase activity in the infants less than 10 hr of age (*above*).

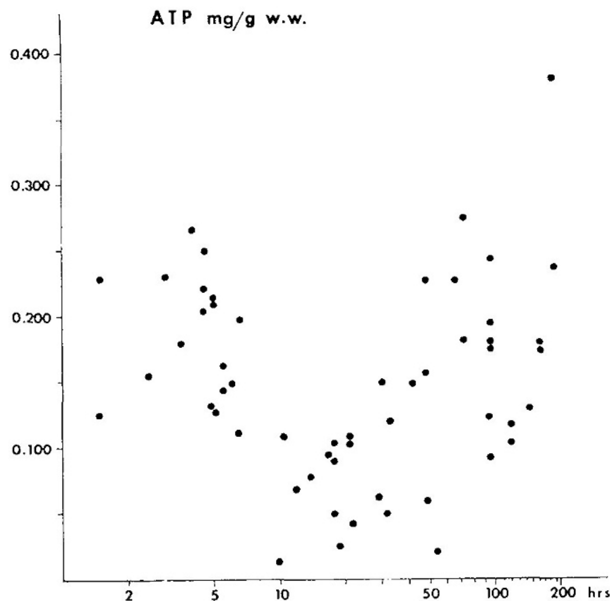

Fig. 3. Age-dependent changes in ATP content (milligrams per gram wet weight) in intact adipose tissue samples from normal human newborn infants in the first 10 days of life.

creased ($P < 0.001$) between 42 and 192 hr of age, 0.171 ± 0.017 ($n = 21$), in comparison with the group 10–33 hr of age.

In the first 24 hr of life ATP content of subcutaneous adipose tissue was inversely correlated with the length of time since the onset of labor. A semilogarithmic relation, $\log y = -0.657 - 0.016x$, $r = 0.469$, $P < 0.01$ ($N = 30$), gives a better fit than a linear one (Fig. 4). A relation between glycogen content and ATP content of adipose tissue can also be seen by comparison of earlier findings [25] with those of this study. The ATP content shows a trend similar to that previously seen for glycerol release and glycerol content of the adipose tissue [22, 25] in the first days of life (Fig. 5).

Discussion

In normal human neonates, enhanced lipid mobilization increases blood levels of free glycerol [20] and free fatty acids [24, 37] in the first hours of life. Dynamics of lipolysis studies *in vitro* in adipose tissue from neonates show that lipolysis increases rapidly from the moment of birth, reaches its maximum within a few hours, and then gradually decreases [22, 25]. The biochemical mechanism of elevated lipid mobilization in human neonates is not known exactly [29, 40], but in newborn lambs activity of the sympathetic nervous system is increased at this time [38]. The basal release of glycerol from adipose cell suspensions has been found to be higher in the first hours of extrauterine life than at any other time during the first 10 days of life, yet response to norepinephrine was less [23, 25].

The low FFA release from subcutaneous adipose tissue immediately after birth can be related to its elevated content of glycogen [25]. The glycogen in adipose tissue is being rapidly metabolized and may moderate free fatty acid release [34] by furnishing increased α-glycerophosphate for re-esterification. Glycogen breakdown certainly begins before delivery and may be initiated at the onset of labor. Previous investigations *in vitro* in subcutaneous adipose tissue with ^{14}C-(U)-D-glucose show increased glucose oxidation and incorporation of glucose carbons into triglyceride immediately after birth, in comparison with older neonates [28].

The increased activity of glycogen phosphorylase in the first hours of life is in agreement with the rapid fall of glycogen in adipose tissue at that time [27]. The development of total and active phosphorylase activity in adipose tissue from human neonates was found to be similar to that in brown fat of the newborn rat [36]. More glucose 1-phosphate is available for the glyco-

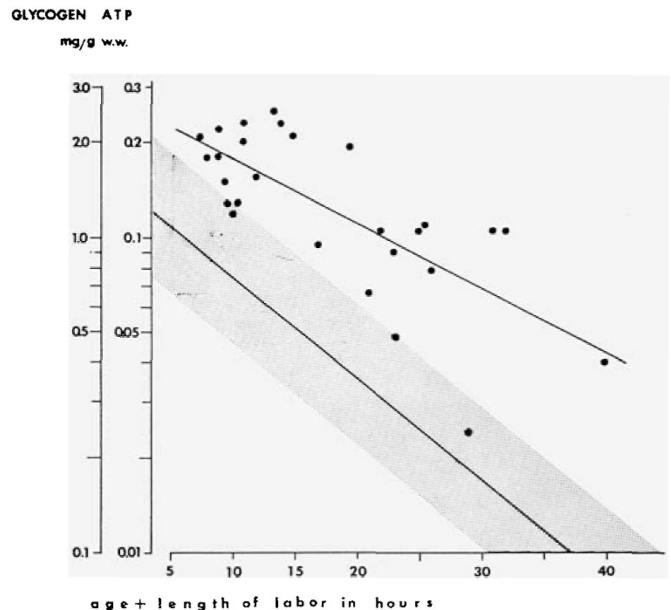

Fig. 4. Comparison of the effect of the length of time since the onset of labor on glycogen content [25] and ATP content of subcutaneous adipose tissue from normal human neonates in the 1st day of life. The *lower line* describes the glycogen content ± SE, $\log y = 0.196 - 0.0316)x$, $r = -0.6671$, $P < 0.0001$ ($n = 29$). The scatter diagram and *upper line* are for the ATP content: $\log y = -0.0164x$, $r = -0.469$, $P < 0.01$ ($n = 30$).

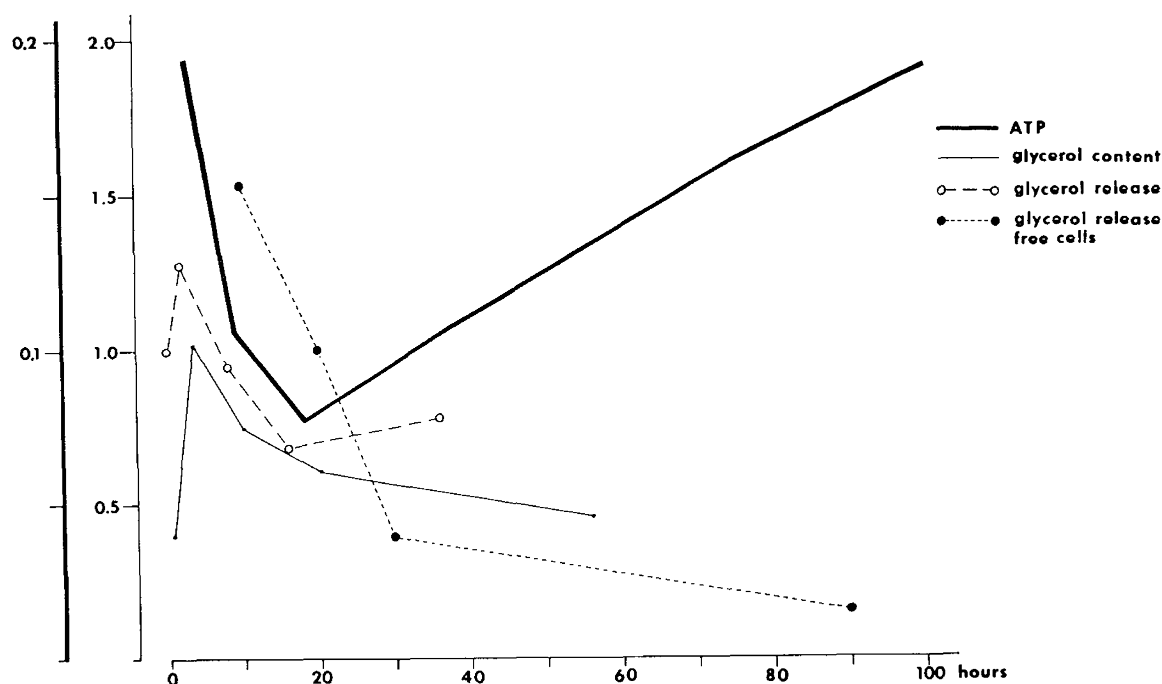

Fig. 5. Age-related changes in mean ATP content of adipose tissue from normal human newborn infants in comparison with glycerol content and glycerol release [22] in intact adipose tissue samples and with glycerol release [25] from suspensions of isolated adipose cells.

lytic pathway in the first hours of life; it may serve as a source of α-glycerophosphate for fatty acid esterification and for oxidation via the Krebs cycle. It is reasonable to hypothesize that increased ATP will be regenerated as long as enough carbohydrate is available for oxidation. On the other hand, the intensive fatty acid re-esterification consumes ATP.

Because both glycogen phosphorylase and triglyceride lipase are activated by cyclic AMP, adenyl cyclase activity should play an important role in mediating the actions of hormones in the adipose tissue. Changes in basal, norepinephrine-stimulated, and fluoride-stimulated adenyl cyclase has been studied by Skala *et al.* [36] in brown adipose tissue from the rat during perinatal and early postnatal development. This group found that the activity of fluoride-stimulated adenyl cyclase increases after birth; similar but less profound changes were observed in basal and norepinephrine-stimulated adenyl cyclase. Although the ultimate activation process by hormones and fluoride appears to be the same, hormones and fluoride apparently act through different mechanisms. Our findings regarding fluoride-stimulated adenyl cyclase in human neonates may represent the maximal capabilities of the tissue to generate cyclic AMP. If so, the positive correlation between the length of time since the onset of labor and adenyl cyclase in the first 10 hr of life suggests that labor triggers the development of adenyl cyclase. Before presenting definite conclusions regarding age-related changes in adenyl cyclase activity in subcutaneous (white) adipose tissue from human neonates, more experiments are needed to determine basal adenyl cyclase activity and the sensitivity of the adenyl cyclase system to different hormones. Such studies could give further insight into the mechanisms responsible for lipid mobilization in the human neonate in the first days of life.

In addition to activating the hydrolysis of triglycerides, lipolytic agents are known to produce a variety of other effects in adipose tissue. One of these is accelerated glucose uptake and rate of conversion to glyceride glycerol as well as increased oxidation of glucose [6, 8, 10, 17]. Only for a short period after birth can increased amounts of intracellular glycogen and extracellular glucose be expected to produce additional α-glycerophosphate in adipose tissue to serve as acceptor of fatty acids for re-esterification; during this time increased amounts of ATP are present to support both lipolysis and fatty acid re-esterification. When calculated from the time of onset of labor, both glycogen and ATP have been observed to decrease exponentially in the adipose tissue in the 1st day of life (Fig.

4). Glycogen content falls somewhat more rapidly than ATP content, which suggests that the fall in glycogen is the primary event and the oxidation of the increased glycogen deposits may be maintaining increased ATP levels in the adipose tissue in the first hours of life.

The 1st day of life represents a period of starvation, yet at the same time, the infant must meet the increased metabolic requirements of extrauterine life. The consumption of food begins at 24 hr of age and increases gradually from this time; synchronously the adipose tissue slowly restores its ATP content.

Comparison of the ATP content of the subcutaneous adipose tissue in human neonates with lipolysis *in vitro* in the 1st week of life reveals closely parallel changes in these variables, except for a short period of accelerated lipolysis after delivery (Fig. 5). The actions of lipolytic agents, whether lipolytic hormones or cyclic AMP, would tend to reduce adipocyte ATP level if no carbohydrate is available to induce a sparing effect. On the other hand, utilizable carbohydrates can enhance hormone-stimulated lipolysis [3, 11, 12]. This was demonstrated in white adipose tissue by Ho [11], who suggested that this effect may be related, at least partially, to improved ATP production.

Adequate stores of glycogen in newborn adipose tissue are important in support of lipolysis and in control of the rate of FFA release, at least during the first hours of extrauterine life. The decrease in ATP content in the subcutaneous adipose tissue may be related to other factors in addition to decreases of substrates for its regeneration. The effects of a lipolytic stimulus on ATP levels are dependent on intracellular accumulation of FFA [2, 32]. The lipolytic process in the fat cell is also regulated by the intracellular concentration of fatty acids [33]. It is known that long chain fatty acids uncouple oxidative phosphorylation in mitochondrial preparation [30], and that this phenomenon can occur in white fat [10]. We have no direct information regarding accumulation of FFA in the intracellular compartment in the adipocyte of the neonate; however, the FFA content of intact subcutaneous adipose tissue fragments from human neonates rises rapidly during the 1st postnatal day [18].

Glucose (1 mg/ml) increases glycerol release significantly in isolated fat cells obtained from newborn infants at the end of the 1st day of life; this increase is greater than that seen after norepinephrine (5 μg/ml) [19]. The cytoplasmic fatty acids are concentrated in the particulate compartments and the process of fatty acid esterification, *i.e.,* the coupling of α-glycerophosphate with acyl-coenzyme A has been described to be localized mainly on the mitochondria [13, 14]. The increased number and complexity of these organelles in subcutaneous fat in the human neonate [26] may be associated with the higher esterification capability of this tissue. More free fatty acids may undergo direct oxidation in neonatal subcutaneous white adipose tissue than in the same tissue in adults [21]. The increase in β-hydroxyacyl-CoA dehydrogenase activity associated with the mitochondrial fraction in subcutaneous adipose tissue during postnatal development suggests such a possibility [28]. There are still insufficient data to confirm or refute the quantitative significance of fatty acid oxidation in developing white adipose tissue in human neonates in comparison with brown fat in human neonates or lower mammals on the one hand, or to white adipose tissue in the human adult on the other hand, where the major function of this tissue is known to be the storage of lipid and the controlled delivery of free fatty acids and glycerol to other organs.

Glycogen stores and their rate of utilization in subcutaneous adipose tissue of newborn infants may be dependent on intrauterine conditions before and during birth. Higher glycogen content as well as increased glycerol release from intact adipose tissue have been found in human neonates who are offspring of diabetic mothers [19]. Accelerated triglyceride turnover, *i.e.,* increased lipolysis with a high esterification rate, seems to be related to higher glycogen content in the adipose tissue of these neonates. On the other hand, anoxia is known to decrease glycogen content in neonatal tissue [35], and has an inhibitory effect on lipolysis [4, 5]. This effect may be related to insufficient oxygen supply for oxidative phosphorylation, which in turn may be reflected in insufficient substrate for ATP-dependent processes involved in lipolysis.

Summary

The metabolism of subcutaneous adipose tissue derived from human neonates of different ages was investigated *in vitro.* In the immediate postnatal period, glycogen phosphorylase activity is increased in comparison with older neonates. Fluoride-stimulated adenyl cyclase activity is directly correlated with the length of time since the onset of labor. The ATP content decreases rapidly in the first hours of life and increases after the 1st day. The changes in phosphorylase activity and ATP content correlate well with the rate of

glycogenolysis and lipolysis found previously in neonatal adipose tissue.

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41. AR, Fischer Scientific Company, Pittsburgh, Pa.
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43. Worthington Biochemical Coorporation, Freehold, N. J. Glucostat was used for the enzymatic determination of glucose.
44. Amersham/Searle Corporation, Arlington Heights, Ill.
45. A. G. Dowex 50-H^+, 100–200 mesh, Bio-Rad Laboratories, Richmond, Calif.
46. Boehringer-Mannheim Corporation, no. 15979 TAAC.
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