Reprinted from THE JOURNAL OF CLINICAL INVESTIGATION, Vol. 49, No. 7, July 1970
Printed in U. S. A.

Carbohydrate Metabolism in Pregnancy

VIII. METABOLISM OF ADIPOSE TISSUE ISOLATED FROM FED AND FASTED PREGNANT RATS DURING LATE GESTATION

ROBERT H. KNOPP, EMILIO HERRERA, and NORBERT FREINKEL

From the Section of Endocrinology and Metabolism, Departments of Medicine and Biochemistry, Northwestern University Medical School, Chicago, Illinois 60611

ACT The effects of late pregnancy on adipose
etabolism have been examined in fed and fasted
mbar fat was excised from 19-day pregnant and
ched virgin rats which had been given un-
d access to food ("fed") or fasted for 48 hr
acrifice.
e fed state, adipose tissue from pregnant rats
d an increased content of free fatty acids (FFA).
incided with augmented cleavage of preformed
es during incubation in vitro as evidenced by
net production of FFA and glycerol, and altered
on of labeled glucose. The enhanced lipolysis
ependent of the availability of glucose and was
mpanied by impaired responsiveness to the anti-
or to the lipogenic actions of added insulin. In
ence of glucose and albumin, esterification as well
sis was greater in adipose tissue from pregnant
ngravid animals. All the differences were exag-
by prior fasting.
properties of adipose tissue during late gesta-
e been ascribed to a primary activation of lipoly-
er than impaired esterification or resistance to
It has been suggested that the hormones of
cy may be responsible. Although increased in-
food and heightened availability of insulin may offset the net lipolytic effects in the fed state, a heightened turnover of adipose stores is always present. Thus, the pregnant animal appears better poised to mobilize preformed fat whenever exogenous nutrients are withheld.

INTRODUCTION

Accelerated mobilization of depot fat in response to fasting has long been recognized as one of the metabolic characteristics of late pregnancy (1). Recent findings suggest that turnover in adipose depots may be altered in the fed state as well. Thus, plasma free fatty acids (FFA) are increased during late gestation in the rat even when access to food is uninterrupted (2–5). These observations, and the paucity of published data concerning adipose tissue in pregnancy, prompted the present studies. Incubations were performed in vitro with lumbar fat from fed and fasted 19-day pregnant, and age-matched nongravid rats. Segments of adipose tissue rather than isolated cells were employed in order to minimize preparative delay, and to preserve the intracellular allosteric and hormonal interrelationships that might be of regulatory significance in vivo.

METHODS

Pregnant primipara and age-matched virgin female rats were secured from Charles River Laboratories, Wilmington, Mass., and housed as in previous studies (5, 6). Experiments were conducted on day 19 of pregnancy (age 60–70 days; fetal weights 1.5–2.0 g). Animals had been given continuing access to Purina Chow pellets ("fed") or deprived of all food but not drinking water for the preceding 48 hr ("fasted"). Rats were maintained in dark animal quarters from 6 p.m. to 8 a.m. each day and sacrificed before 11 a.m. Pregnant animals with litters of less than eight fetuses were excluded.

Right and left lumbar fat pads were excised, and two

f this work was presented at the 51st Annual Meet-
ie Endocrine Society, New York, 27–29 June 1969
t No. 6).
iopp is a U. S. Public Health Service Trainee in
ology and Metabolism, 1966–1968. His present
U. S. Public Health Service, Diabetes and Arth-
n, Boston, Mass. Dr. Herrera is a Research Fel-
Endocrinology and Metabolism, 1965–1968. His
ddress is Consejo Superior de Investigaciones
Instituto "G. Marañon," Velazquez 144, Madrid 6,

ıg pieces, one from each side, were introduced into ials containing 2 ml of either one of the following on media: (*a*) KRB: modified Krebs-Ringer-bi-te containing gelatin (<2 mg/ml) as per Bail, and Cooper (7) to prevent absorption of insulin to re, or (*b*) KRB-Alb: modified Krebs-Ringer-Bi-te containing approximately 0.4 mM (i.e. 28 mg/ml) (Armour: Bovine albumin) which had been treated ıarcoal adsorption (8) and extensive dialysis in oratory to remove free fatty acids (FFA) and or-cids. Heptane extracts of 10-mg aliquots of such albumin contained an average of 0.030 μmoles titrata-. Insulin and glucose were added to KRB or KRB- described in the text. The insulin contained "less 05% glucagon" and was generously supplied by Dr. Root of Eli Lilly & Co. Epinephrine was prepared ıg to Hagen and Ball (9), diluted 1000-fold in KRB ıtely before use, and added to a final concentration ug/ml. Preparations of glucose labeled in carbon-1 glucose-1-^{14}C; glucose-6-^{14}C) were purchased from ıgland Nuclear Co.; 0.5 or 1.0 μCi was added to in-vessels, the larger amounts being used with tissues ıted rats.

ls were sealed with rubber caps, gassed with 95% CO_2, and incubated at 38°C in a Dubnoff shaker as d previously (10, 11). For experiments with la-ucose, $^{14}CO_2$ was collected as before (11); total lipids tracted by the method of Folch, Lees, and Sloane-(12) and washed twice with saline. Lipid extracts ponified (1 hr; 80°C; 5 N methanolic KOH) and oactivity was partitioned into fatty acids and glycer-erol (13). Radioactive standards were prepared from which had been incubated without tissues.

-ol concentrations in tissues and media were measured ically (14); recovery of glycerol added to tissue ex-xceeded 90%. FFA were extracted from tissue and s per Dole and Meinertz (15); the heptane extracts luced to dryness under N_2 and resuspended in chloro-.ctivated silicic acid was added to the chloroform ve phospholipids as described elsewhere (5), and re estimated by the Duncombe procedure (16). The pellet after lipid extraction of tissues was employed ate total tissue protein by the procedure of Lowry, ugh, Farr, and Randall (17) and DNA-phosphorus nethod of Schmidt and Thannhauser (18). Tissue was employed as a reasonable index of functional e mass (19) and all measurements were expressed gram tissue protein.[1]

For statistical analyses, unpaired data were compared using the student *t* test. Paired data were evaluated for statistical significance by assessing whether the ratios of "experimental" to "control" values deviated significantly from unity. Student *t* tests were performed after logarithmic transformation of the ratios (21).

RESULTS

Adipose tissue from fed animals

Relationships In Vivo

Elsewhere (4, 5), we have confirmed that plasma glucose and ketones are lower (3, 22), whereas FFA are higher (2, 3) in fed 19-day pregnant than in age-matched virgin rats. We have also reported that plasma immunoreactive insulin is almost doubled in fed gravid vs. virgin animals (4, 5). To assess whether the elevations of plasma FFA truly coincide with an increased availability of FFA from adipose tissue stores, portions of lumbar fat from animals given continuing access to food were excised rapidly, frozen in liquid N_2, and analyzed for glycerol and FFA. As shown in Table I, the tissue content of FFA was substantially greater in fat from fed pregnant than virgin rats ($P < 0.02$).[2] Values for tissue glycerol were low and not significantly different in tissues from pregnant and virgin animals (Table I).

Metabolism In Vitro

The augmented tissue FFA of the pregnant (as cf. virgin) rats in the fed state could result from: (*a*) a primary defect in esterification due directly to the lower plasma glucose, or indirectly to diminished effectiveness of insulin upon glucose utilization; or (*b*) a primary increase in lipolysis due directly to increased availability of a lipolytic agent, or indirectly to diminished effectiveness of insulin upon restraining triglyceride lipase; or (*c*) "carry-over" into fed state of the "accelerated starvation" (1) that could occur between feedings during

weights of lumbar fat pads were about 50% greater 19-day pregnant than in age-matched virgin rats. NA-phosphorus within fat pads was not significantly (17.9 ±2.5 vs. 17.1 ±1.6 μg DNA-phosphorus per from pregnant and virgin animals respectively) glycerides were more abundant in the pregnant ±6.6 vs. 153.0 ±3.0 μmoles esterified fatty acids per A-phosphorus; $P < 0.001$). Thus, much of the dif-in weight appears to be due to the amount of fat per er than the number of fat cells per fat pad. In keep-this conclusion, we encountered substantially lower ations of protein (i.e. mg protein/g wet weight) in ar adipose tissue from fed pregnant animals (11.9 17.4 ±0.1; $P < 0.001$). Relative differences in pro-centrations were even greater after 48 hr fast (i.e. vs. 27.0 ±3.2 mg protein/g lumbar adipose tissue ant vs. virgin respectively; $P < 0.001$). However, tened vascularity and proportionately greater con-

tributions from occluded blood (20) may render protein concentration a less reliable index of the functional mass of the fat cells within adipose tissue after fasting.

[2] Ballard and Hanson have estimated "glucose space" in epididymal fat from fed and 72 hr fasted rats, and employed those measurements, and concurrent analyses of plasma to correct for extracellular contributions to the apparent concentrations of metabolites in adipose tissue in vivo (20). The tissue concentrations of FFA shown in Table I sufficiently exceeded those of plasma FFA that they were not altered meaningfully when corrected on the basis of the average reported "glucose spaces" (20) (i.e., 4.90% and 7.42% of total adipose tissue space in fed and fasted animals respectively) and the average plasma FFA which we have observed in fed and 48 hr fasted virgin and 19-day pregnant rats (5). Corrected mean ±SEM values for adipose tissue content of FFA (μmoles/mg tissue protein) in pregnant vs. virgin rats were: 0.269 ±0.040 vs. 0.154 ±0.024 ($P < 0.05$) in fed and 0.650 ±0.038 vs. 0.238 ±0.015 ($P < 0.001$) in fasted animals respectively.

TABLE I

*Effect of Pregnancy on Adipose Tissue Content of FFA and Glycerol**

	FFA	Glycerol
	μmoles/mg tissue protein	
rats		
iant	0.258 ±0.038 (6)	0.012 ±0.001 (6)
n	0.147 ±0.023 (8)	0.011 ±0.002 (6)
	<0.02	NS
ed rats		
iant	0.560 ±0.036 (12)	0.036 ±0.005 (8)
n	0.222 ±0.014 (8)	0.020 ±0.002 (12)
	<0.001	<0.01

nts of lumbar fat were rapidly excised from 19-day it and age-matched virgin rats which had been given rupted access to food ("fed") or deprived of food for "fasted") prior to sacrifice. Mean ±SEM values are bove; ( ) denotes number of animals; *P* indicates sig- e of differences between values in pregnant and virgin S = not significant.

station. To distinguish among these possibilities, ts of lumbar fat from fed pregnant and virgin re incubated under conditions which eliminated erences in the concentration of plasma glucose to hey are exposed in vivo.

ation and esterification of labeled glucose. Dur- ubation for 60 min in KRB containing 5 mM glucose labeled in carbon-1 or -6, oxidation of carbon-6 was greater by adipose tissue from fed pregnant than fed virgin rats ($P < 0.02$), and more labeled glyceride-glycerol was formed from both carbon-1 ($P < 0.05$) and carbon-6 ($P < 0.05$) (Table II).

With epididymal fat, analogous patterns have been elicited by the addition of lipolytic agents in vitro (10, 23–27). Thus, the increases in carbon-6 oxidation and glyceride-glycerol formation could be compatible with antecedent activation of lipolysis in vivo. However, since such changes in glucose metabolism have also been effected by simply increasing tissue FFA (10, 23, 25), the phenomena might merely reflect the higher initial intracellular FFA (Table I). Accordingly, more direct assessments of fat turnover were secured during longer incubations, *in the presence of albumin as an extracellular FFA-acceptor.*

Net release of FFA and glycerol in albumin-containing systems. Mean ±SEM values for FFA and glycerol following incubation for 150 min are summarized in Table III.[a]

In KRB-Alb, adipose tissue from fed pregnant rats

[a] The Table depicts final values for the FFA within tissues, and the FFA and glycerol within media. Final concentrations of tissue glycerol were estimated in separate experiments. During 150 min incubation, tissue glycerol invariably declined below initial levels (Table I) and did not correlate with the net release of glycerol into the incubation media. Therefore, for economy of space, final tissue glycerol has been omitted from Tables III, IV, and VI although the values were employed to calculate "net lipolysis" and "net esterification" (*vide supra*).

TABLE II

*Effects of Pregnancy on Oxidation and Esterification of Labeled Glucose by Adipose Tissue In Vitro**

	$^{14}CO_2$		Glyceride-glycerol-^{14}C	
	Glucose-1-^{14}C	Glucose-6-^{14}C	Glucose-1-^{14}C	Glucose-6-^{14}C
	mμmoles of glucose carbon/mg tissue protein			
A. Fed Rats				
Pregnant (6)	33.7 ±3.5	16.4 ±2.4	45.8 ±7.9	37.6 ±4.3
Virgin (6)	32.1 ±4.2	9.5 ±0.8	29.2 ±3.3	26.5 ±2.1
P	NS	<0.02	<0.05	<0.05
B. Fasted Rats				
Pregnant (5)	20.2 ±3.1	15.3 ±0.7	50.7 ±5.8	29.9 ±2.1
Virgin (6)	8.5 ±0.5	4.6 ±0.6	19.7 ±1.9	12.8 ±1.5
P	<0.01	<0.001	<0.001	<0.001

* Tissues were incubated 60 min in 2 ml KRB containing 5 mM glucose-1-^{14}C or glucose-6-^{14}C. Mean ±SEM values for the evolution of $^{14}CO_2$ and formation of glyceride-glycerol-^{14}C have been expressed on the basis of mμmoles of glucose carbon. ( ) denotes the number of animals in each category; *P* indicates significance of differences between values for tissues from pregnant and virgin rats.

TABLE III

*Effects of Pregnancy on Lipolysis and Esterification by Adipose Tissue during Incubation in Albumin-Containing Media: Fed Rats**

	KRB-Alb	KRB-Alb +glucose	P‡	KRB-Alb + glucose +insulin	P‡
		μmoles/mg tissue protein			*μmoles/mg tissue protein*
Final medium glycerol					
Pregnant	0.151 ±0.019	0.217 ±0.019	<0.01	0.134 ±0.015	<0.001
Virgin	0.094 ±0.008	0.148 ±0.010	<0.01	0.089 ±0.013	<0.001
P§	<0.02	<0.01		<0.05	
Final medium FFA					
Pregnant	0.235 ±0.019	0.167 ±0.029	<0.01	0.035 ±0.022	<0.001
Virgin	0.124 ±0.019	0.092 ±0.022	NS	0.022 ±0.015	<0.001
P	<0.01	<0.05		NS	
Final tissue FFA					
Pregnant	0.218 ±0.020	0.175 ±0.031	<0.01	0.152 ±0.018	NS
Virgin	0.127 ±0.007	0.118 ±0.010	NS	0.092 ±0.013	<0.05
P	<0.01	NS		<0.02	
Net lipolysis					
Pregnant	0.435 ±0.056	0.663 ±0.054	<0.001	0.398 ±0.043	<0.001
Virgin	0.263 ±0.026	0.416 ±0.029	<0.01	0.250 ±0.041	<0.001
P	<0.02	<0.01		<0.05	
Net esterification					
Pregnant	0.206 ±0.039	0.545 ±0.045	<0.001	0.435 ±0.066	<0.05
Virgin	0.148 ±0.024	0.342 ±0.029	<0.01	0.272 ±0.027	<0.05
P	NS	<0.01		<0.05	

* Tissues from fed 19-day pregnant (n = 6) and age-matched virgin (n = 6) rats were incubated 150 min in KRB containing approximately 0.4 mM albumin (KRB-Alb). Effects of including 3.75 mM glucose (KRB-Alb + glucose) or 3.75 mM glucose plus 50 μU/ml insulin (KRB-Alb + glucose + insulin) in the incubation medium were evaluated. The table summarizes mean ±SEM values for FFA within tissue and for FFA and glycerol within medium at the end of incubation in terms of μmoles/mg tissue protein. Mean ±SEM values for net lipolysis and esterification (μmolesFFA/mg tissue protein) during incubation were derived as per Vaughan (28).

‡ *P* denotes significance of the effects of glucose (i.e. KRB-Alb vs. KRB-Alb + glucose) or insulin (i.e. KRB-Alb + glucose vs. KRB-Alb + glucose + insulin) within each group of animals.

§ *P* denotes significance of the effects of pregnancy by comparing pregnant vs. virgin animals.

l considerably more FFA than did tissue from gin rats ($P < 0.01$). Tissue FFA at the end of ion (Table III) persisted near levels found ini- Table I) so that the efflux represented greater ion of FFA rather than simple depletion of more it tissue stores. Indeed, derived values for the duction of FFA in KRB-Alb (i.e. final FFA [me- tissue] − initial tissue FFA) were 0.229 ±0.038 5 ±0.026 μmoles/mg protein for the tissues from t vs. virgin animals respectively ($P < 0.05$). sion of 3.75 mM glucose (i.e. KRB-Alb + glu- able III) obtunded the net release of FFA. r, as evidenced by the greater release of glycerol 01), the tissues from gravid animals continued more glycerides.

The measurements of glycerol and FFA were combined to derive estimates of lipolysis and esterification as per Vaughan (28) (Table III). It was felt that the limited glycerokinase activity in white adiopse tissue (29), and the more recent direct documentation that glycerol is reutilized by epididymal fat in vitro,[4] would not preclude application of the Vaughan calculations (28) to assess *net changes during the period of incubation* (30), and *to compare* pregnant vs. nongravid animals thereby. As estimated in this fashion (28, 30), *net* lipolysis during incubation in KRB-Alb was about 60% greater in tissues from pregnant animals ($P <$

[4] Herrera, E., A. Ayanz, and L. Lamas. 1968–1969. Unpublished observations.

TABLE IV

*Effects of Pregnancy on the Responsiveness of Adipose Tissue to Inhibition of Lipolysis by Insulin**

	KRB	KRB + insulin	P‡	KRB + epinephrine	KRB + epinephrine + insulin	P‡
	μmoles/mg tissue protein			*μmoles/mg tissue protein*		
inal medium glycerol						
Pregnant	0.079 ±0.013	0.063 ±0.008	<0.01	0.161 ±0.019	0.107 ±0.020	<0.01
Virgin	0.076 ±0.006	0.063 ±0.005	<0.001	0.152 ±0.015	0.109 ±0.018	<0.01
P§	NS	NS		NS	NS	
inal tissue FFA						
Pregnant	0.327 ±0.050	0.305 ±0.045	NS	0.581 ±0.065	0.416 ±0.065	<0.001
Virgin	0.192 ±0.021	0.155 ±0.011	<0.02	0.404 ±0.045	0.236 ±0.046	<0.001
P	<0.05	<0.01		<0.05	<0.05	
et lipolysis						
Pregnant	0.209 ±0.037	0.164 ±0.026	<0.01	0.456 ±0.059	0.300 ±0.062	<0.01
Virgin	0.200 ±0.018	0.160 ±0.016	<0.001	0.428 ±0.045	0.298 ±0.048	<0.001
P	NS	NS		NS	NS	
et esterification						
Pregnant	0.131 ±0.021	0.136 ±0.018	NS	0.118 ±0.021	0.142 ±0.026	NS
Virgin	0.157 ±0.024	0.151 ±0.030	NS	0.172 ±0.024	0.176 ±0.027	NS
P	NS	NS		NS	NS	

ies from fed 19-day pregnant (n = 6) and age-matched vrigin (n = 8) rats were incubated 150 min in KRB. The table arizes mean ±SEM values for FFA within tissue and for glycerol within medium at the end of incubation. (In the KRB is, no FFA were released into the medium.) Mean ±SEM values for net lipolysis and esterification were derived as per an (28).

notes significance of the effects of insulin on basal (KRB vs. KRB + insulin) or stimulated (KRB + epinephrine vs + epinephrine + insulin) lipolysis within each group of animals.

notes significance of the effects of pregnancy by comparing pregnant vs. virgin animals.

whereas *net* esterification did not differ in the roups (Table III). During incubation in KRB-Alb cose, the removal of restraining amounts of FFA erification (31) enabled more lipolysis to occur h groups (Table III). However, net lipolysis re- d about 60% greater in the tissues from the gravid ls ($P < 0.01$) coincident with an equally greater terification ($P < 0.01$).

is, every aspect of fat turnover is augmented in e tissues from the fed pregnant animals, and ened lipolysis rather than impaired esterification responsible for the enhanced efflux of FFA.

ponsiveness to insulin. The effects of insulin were ned to assess whether resistance to insulin action be implicated in these phenomena.

shown in Table III, addition of 50 μU/ml insulin B-Alb + glucose media dampened FFA and glyc- lease from adipose tissue of pregnant as well as in vitro. On a percentile basis, the reductions of olysis by insulin (i.e. KRB-Alb + glucose vs. Alb + glucose + insulin) were not different in groups: 37.7 ±8.7% in the pregnant and 41.1 virgin.

Similar results were obtained during 150-min incubations in simple KRB systems (Table IV). In the absence of either glucose to facilitate esterification, or albumin to permit efflux of tissue FFA, differences in basal lipolysis between tissues from pregnant and virgin animals could not be demonstrated (Table IV) presumably because the higher initial tissue FFA (Table I) inhibited full lipolysis (31) in the pregnant rats. However, addition of 50 μU/ml insulin obtunded basal as well as epinephrine-stimulated lipolysis to an equal degree in both groups (Table IV). Thus, isolated adipose tissue from fed pregnant rats did not display absolute or relative resistance to the antilipolytic effects of insulin under any experimental situation.

To evaluate tissue sensitivity to purely anabolic actions of insulin, formation of labeled fatty acids was examined. Table V summarizes results obtained during incubation of adipose tissue from fed animals for 60 min in KRB containing 5 mM glucose-1-^{14}C or glucose-6-^{14}C. Net biosynthesis of fatty acids in vitro by segments of lumbar fat from pregnant and virgin rats was not significantly different in the absence of added insulin ("0 insulin") nor in the presence of submaximal (100 μU/

TABLE V

*Effect of Pregnancy on the Formation of Fatty Acids from Labeled Glucose by Adipose Tissue In Vitro**

	Tissue fatty acid-^{14}C *mμmoles of glucose carbon/mg tissue protein*					
μU/ml...	0		100		1000	
	Glucose-1-^{14}C	Glucose-6-^{14}C	Glucose-1-^{14}C	Glucose-6-^{14}C	Glucose-1-^{14}C	Glucose-6-^{14}C
Rats						
nant (6)	11.6 ±2.4	18.2 ±3.9	33.4 ±6.3	68.1 ±18.1	69.1 ±11.3	212.0 ±61.8
in (6)	13.2 ±2.9	12.2 ±2.4	21.6 ±4.0	48.5 ±6.0	42.6 ±7.8	118.6 ±19.1
	NS	NS	NS	NS	NS	NS
ted Rats						
nant (5)	0.20 ±0.05	0.25 ±0.05	0.50 ±0.20	1.60 ±1.00	7.10 ±5.50	14.5 ±11.0
in (6)	0.16 ±0.04	0.13 ±0.04	1.00 ±0.30	1.70 ±0.60	4.50 ±1.80	8.6 ±4.0
	NS	NS	NS	NS	NS	NS

ies were incubated 60 min in 2 ml KRB containing 5 mM glucose-1-^{14}C or glucose-6-^{14}C; and 0, 100, or 1000 μU/ml insulin.

or maximal (1000 μU/ml) insulin stimulation e V).

h epididymal fat, the recovery of radioactivity in fatty acids, and glyceride-glycerol has accounted -90% of glucose assimilation in vitro (24, 32). By riterion, glucose "uptake" was about one-third r in adipose tissue from pregnant than virgin rats n the *absence of added insulin* (Tables II and V). relationships can be extrapolated to events in vivo, ıld appear that the lower plasma glucose in the egnant rat (3–5, 22) is not attended by glucose ation in adipose tissue; and that adipose tissue is volved in the diminished hypoglycemic response to ı (33). Thus, not only is responsiveness to in- preserved, but metabolism is geared to compensate e prevailing lower concentration of extracellular e.

ipose tissue from 48-hr fasted animals

ATIONSHIPS IN VIVO

lowing a 48 hr fast, plasma glucose falls to near lycemic levels in the 19-day pregnant rat (3–5, plasma insulin ceases to be greater than in non- l animals (4, 5) and urinary catecholamine ex- n is increased (6) (whereas urinary catecholamines ıaffected by pregnancy when food is available [6]). ident (and perhaps associated) with these changes, perlipacidemia (elevated FFA levels) and ketone- f starvation are markedly exaggerated in the preg- at (3–5, 22). Table I summarizes values for the e tissue content of FFA and glycerol under these stances. Although the 48 hr fast increased tissue and glycerol in nongravid as well as gravid ani- the increments for both were 2.7-fold greater in egnant group. Thus, FFA were approximately doubled in the adipose tissue of fasted pregnant rats but increased only about 50% in the virgin (Table I).

The divergences in vivo prompted repeat studies in vitro. It was felt that prior fast should enhance the in vitro differences between tissues from fed pregnant and nonpregnant animals which we have ascribed to gestational activation of lipolysis (*vide supra*).

METABOLISM IN VITRO

The per cent of total radioactivity per milligram tissue protein which was recoverable as $^{14}CO_2$ or glyceride-glycerol-^{14}C after 60 min incubation in KRB was diminished by fasting (Table II). However, the absolute differences in glucose oxidation and esterification between tissues from gravid and nongravid animals were, if anything, more pronounced.

During more prolonged incubation in KRB-Alb (Table VI) both groups released greater amounts of FFA and glycerol into the medium than in the fed state (Table III). However, the disparities between pregnant and virgin animals were even more marked following 48 hr fast (Table VI). Inclusion of glucose (i.e. KRB-Alb + glucose) effected proportionally smaller increases in net esterification after fasting (Table VI) than in the fed state (Table III) but caused even greater differences in net lipolysis between the virgin and pregnant rats (Table VI). Under such circumstances, addition of insulin (50 μU/ml) reduced net lipolysis 10.1 ±4.1% in pregnant ($P < 0.05$) and 31.4 ±1.4% in virgin ($P < 0.001$) (Table VI)—a significant difference between the two groups ($P < 0.01$).

Fasting also did not alter the relative responsiveness to the effects of insulin upon glucose disposition. As shown in Table V, the decreased formation of fatty acids after 48 hr fast was minimally but equally in-

TABLE VI

*Effect of Pregnancy on Lipolysis and Esterification by Adipose Tissue during Incubation in Albumin-Containing Media: Fasted Rats**

	KRB-Alb	KRB-Alb + glucose	*P*	KRB-Alb + glucose + insulin	*P*
	μmoles/mg tissue protein			*μmoles/mg tissue protein*	
Final medium glycerol					
Pregnant	0.286 ±0.026	0.350 ±0.011	<0.01	0.315 ±0.008	<0.05
Virgin	0.148 ±0.013	0.179 ±0.020	<0.02	0.128 ±0.013	<0.001
P	<0.01	<0.001		<0.001	
Final medium FFA					
Pregnant	0.444 ±0.058	0.568 ±0.030	NS	0.364 ±0.062	<0.05
Virgin	0.223 ±0.020	0.226 ±0.041	NS	0.054 ±0.032	<0.02
P	<0.01	<0.001		<0.01	
Final tissue FFA					
Pregnant	0.527 ±0.088	0.361 ±0.028	<0.05	0.338 ±0.019	NS
Virgin	0.147 ±0.010	0.153 ±0.017	NS	0.132 ±0.025	NS
P	<0.01	<0.001		<0.001	
Net lipolysis					
Pregnant	0.790 ±0.082	0.987 ±0.031	<0.02	0.883 ±0.029	<0.05
Virgin	0.420 ±0.040	0.529 ±0.060	<0.01	0.366 ±0.042	<0.001
P	<0.01	<0.001		<0.001	
Net esterification					
Pregnant	0.445 ±0.123	0.684 ±0.029	<0.01	0.808 ±0.065	<0.05
Virgin	0.259 ±0.026	0.358 ±0.037	<0.001	0.387 ±0.037	NS
P	NS	<0.001		<0.001	

* Tissues from fasted 19-day pregnant (n = 5) and age-matched virgin (n = 6) rats were incubated 150 min in KRB-Alb. Experimental conditions, presentation of data, and statistical analyses are as in Table III.

by insulin in tissues from pregnant as well as nimals.

DISCUSSION

ned availability of FFA within adipose tissue ed 19-day pregnant rat has been documented in ent studies. It has been shown that this coincides augmented cleavage of stored triglycerides dur- ubation in vitro as evidenced by greater net ion of FFA and glycerol and altered metabolism ed glucose. The enhanced lipolysis was inde- of glucose availability, occurring in the absence as the presence of glucose. No impairment in olic actions of insulin was discerned as judged effects upon the formation of fatty acids from glucose. The antilipolytic effectiveness of in- s likewise maintained and could not be impli- the presence of glucose and albumin, esterifica- well as lipolysis was greater in adipose tissue gnant than nongravid rats indicating increased tissue lipids analogous to that observed after incubating epididymal fat with a variety of lipolytic agents (30). In other words, isolated fat from the fed 19-day pregnant rat seems to behave as if subjected to a primary stimulation of lipolysis rather than an impairment of esterification or resistance to insulin.

In the light of these findings, the increase in glucose uptake and net gas exchange produced by insulin in adipose tissue from fed 18- to 20-day pregnant rats (34, 35) more likely results from the greater esterification and glucose oxidation than heightened sensitivity to insulin. Likewise, the greater total labeling of maternal fat after intravenous glucose-^{14}C (36) may represent an increment in glyceride-glycerol rather than fatty acid synthesis. Indeed, Fain and Scow found that the incorporation of tritiated water into maternal fatty acids in the 20-day fed pregnant rat was indistinguishable from that of virgin control animals although fatty acid synthesis was substantially greater in the 16-day pregnant animal (22).

Whether the intrinsic activation of lipolysis in adipose tissue fully accounts for the observed elevation of

ma FFA in the fed pregnant rat cannot be answered. er factors such as the greater total body fat (22), lower plasma glucose (3–5, 22), the lower plasma min (37), and the heightened cardiac output (38), possibly augmented perfusion of adipose tissue d also be contributory in vivo. Some of the FFA at even originate from the hypertriglyceridemia of ary origin (3).

he precise reasons for the activation of lipolysis in ose tissue in the fed state are also unclear. We have onstrated that the disparities in lipolysis between ar fat from gravid and nongravid animals are acuated by fasting. Indeed, after 48 hr fast in pregy, some added resistance to insulin may even be ifest as evidenced by diminished responsiveness to the ipolytic actions of the hormone in vitro. Thus, the ibility that some adaptations to the "exaggerated" ation of pregnancy (1, 4, 5) are carried over the fed state cannot be excluded. However, it does seem likely that this is of major importance since r catecholamine excretion is not different in pregthat in nonpregnant animals during ad lib. feeding and it is our impression that pregnant rats not only nore (3) but also more frequently and *throughout day* in late gestation.[8] It seems more likely that the ained activation of lypolysis is triggered by somey that acts continuously. The hormones of pregnancy l qualify for such a role since they are released from nta and ovary without dietary feedback regulation in accord with the growth and development of the eptus (39, 40). One such hormone, placental lactohas displayed lipolytic properties *in vitro* (27) alh the role of placental lactogen is less clear in the han in primates (see reference 5 for review of ature).

uring the normal course of pregnancy, where the er has unlimited access to food, the lipolytic acon need little effect the tendency to increase adipose e mass. Our data would suggest that any putarestraint to fatty acid synthesis is apparently overby the increase in plasma insulin. In addition, duronjoint availability of glucose and insulin, most of atty acids liberated by lipolysis are recovered by concurrent increase in esterification. The cost to ssue may be measured in terms of a small net FFA lus the energy required for esterification (41), the t in fatty acids apparently being more than comted by lipid of dietary or hepatic origin.

lipolytic activation appears established at limited the animal, what useful purpose might it serve? sonable hypothesis would envision the increase in stores, and the coexistent increase in fat turnover as "insurance" against possible food deprivation, particularly in late gestation when the fetal drains on maternal glucose and amino acids are greatest. Should fasting supervene, the pregnant animal is ideally poised to mobilize and utilize lipids at an accelerated rate, and thereby to spare nonlipid nutrients for fetal growth and for maternal tissues which will not accept other fuels.

[8] Knopp, R. H., E. Herrera, and N. Freinkel. Unpublished observations.

ACKNOWLEDGMENTS

We are indebted to Mrs. Susan Tuckett, Miss Jayne Martin, and Miss Dorothy Turek for excellent technical assistance.

This work was supported in part by Research Grant AM-10699 and Training Grant AM-05071 from the National Institute of Arthritis and Metabolic Diseases, U. S Public Health Service, Bethesda, Md.

REFERENCES

1. Freinkel, N. 1969. Homeostatic factors in fetal carbohydrate metabolism. *In* Fetal Homeostasis. Vol. 4. R. M. Wynn, editor. Appleton-Century-Crofts Inc., New York. 85.
2. McKay, D. G., and H. Kaunitz. 1963. Studies of the generalized Schwartzman reaction induced by diet. VI. Effects of pregnancy on iipid composition of serum and tissues. *Metabolism.* **12**: 990.
3. Scow, R. O., S. S. Chernick, and M. S. Brinley. 1964. Hyperlipemia and ketosis in the pregnant rat. *Amer. J. Physiol.* **206**: 796.
4. Freinkel, N., E. Herrera, R. H. Knopp, and H. J. Ruder. 1969. Metabolic realignments in late pregnancy: a clue to diabetogenesis? *In* Early Diabetes. R. A. Camerini-Davalos, editor. Academic Press Inc., New York. 205.
5. Herrera, E., R. H. Knopp, and N. Freinkel. 1969. Carbohydrate metabolism in pregnancy. VI. Plasma fuels, insulin, liver composition, gluconcogenesis, and nitrogen metabolism during late gestation in the fed and fasted rat. *J. Clin. Invest.* **48**: 2260.
6. Herrera, E., R. H. Knopp, and N. Freinkel. 1969. Urinary excretion of epinephrine and norepinephrine during fasting in late pregnancy in the rat. *Endocrinology.* **84**: 447.
7. Ball, E. G., D. B. Martin, and O. Cooper. 1959. Studies on the metabolism of adipose tissue. I. The effect of insulin on glucose utilization as measured by the manometric determination of carbon dioxide output. *J. Biol. Chem.* **234**: 774.
8. Chen, R. F. 1967. Removal of fatty acids from serum albumin by charcoal treatment. *J. Biol. Chem.* **242**: 173.
9. Hagen, J. H., and E. G. Ball. 1960. Studies on the metabolism of adipose tissue. IV. The effect of insulin and adrenaline on glucose utilization, lactate production, and net gas exchange. *J. Biol. Chem.* **235**: 1545.
10. Freinkel, N. 1961. Extrathyroidal actions of pituitary thyrotropin: effects on the carbohydrate, lipid and respiratory metabolism of rat adipose tissue. *J. Clin. Invest.* **40**: 476.
11. Freinkel, N., A. K. Cohen, R. A. Arky, and A. E. Foster. 1965. Alcohol hypoglycemia. II. A postulated mechanism of action based on experiments with rat liver slices. *J. Clin. Endocrinol.* **25**: 76.
12. Folch, J., M. Lees, and G. H. Sloane Stanley. 1957. A simple method for the isolation and purification of total lipides from animal tissues. *J. Biol. Chem.* 226: 497.

ughan, M. 1961. Effect of hormones on glucose tabolism in adipose tissue. *J. Biol. Chem.* **236**: 2196.
rland, P. B., and P. J. Randle. 1962. A rapid enzytic assay for glycerol. *Nature (London).* **196**: 987.
le, V. P., and H. Meinertz. 1960. Microdetermination long-chain fatty acids in plasma and tissues. *J. Biol. cm.* **235**: 2595.
ncombe, W. G. 1964. The colorimetric micro-determiion of non-esterified fatty acids in plasma. *Clin. im. Acta.* **9**: 122.
wry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. ndall. 1951. Protein measurement with the Folin nol reagent. *J. Biol. Chem.* **193**: 265.
midt, G., and S. J. Thannhauser. 1945. A method the determination of deoxyribonucleic acid, ribo-leic acid, and phosphoproteins in animal tissues. *Biol. Chem.* **161**: 83.
inegrad, A. I., and A. E. Renold. 1958. Studies on rat pose tissue *in vitro*. I. Effects of insulin on the tabolism of glucose, pyruvate, and acetate. *J. Biol. cm.* **233**: 267.
llard, F. J., and R. W. Hanson. 1969. Measurements adipose-tissue metabolites *in vivo*. *Biochem. J.* **112**:
inkel, N. 1960. Further observations concerning the ion of pituitary thyrotropin on the intermediate tabolism of sheep thyroid tissue *in vitro*. *Endonology.* **66**: 851.
in, J. N., and R. O. Scow. 1966. Fatty acid synthesis *vivo* in maternal and fetal tissues in the rat. *Amer. hysiol.* **210**: 19.
hill, G. F., Jr., B. Leboeuf, and R. B. Flinn. 1960. idies on rat adipose tissue *in vitro*. VI. Effect of nephrine on glucose metabolism. *J. Biol. Chem.* **235**: 6.
an, W. S., R. M. MacLeod, and R. H. Brown. 1960. ects of epinephrine, insulin, and corticotrophin on the abolism of rat adipose tissue. *J. Biol. Chem.* **235**: 4.
oeuf, B., and G. F. Cahill, Jr. 1961. Studies on rat pose tissue *in vitro*. VIII. Effect of preparations of itary adrenocorticotropic and growth hormones on cose metabolism. *J. Biol. Chem.* **236**: 41.
tz, J., B. R. Landau, and G. E. Bartsch. 1966. The tose cycle, triose phosphate isomerization, and lipoesis in rat adipose tissue. *J. Biol. Chem.* **241**: 727.
rtle, J. R., and D. M. Kipnis. 1967. The lipolytic ion of human placental lactogen on isolated fat cells. *chim. Biophys. Acta.* **144**: 583.
28. Vaughan, M. 1962. The production and release of glycerol by adipose tissue incubated *in vitro*. *J. Biol. Chem.* **237**: 3354.
29. Robinson, J., and E. A. Newsholme. 1967. Glycerol kinase activities in rat heart and adipose tissue. *Biochem. J.* **104**: 2 c.
30. Vaughan, M., and D. Steinberg. 1963. Effect of hormones on lipolysis and esterification of free fatty acids during incubation of adipose tissue *in vitro*. *J. Lipid Res.* **4**: 193.
31. Rodbell, M. 1965. Modulation of lipolysis in adipose tissue by fatty acid concentration in fat cell. *Ann. N. Y. Acad. Sci.* **131**: 302.
32. Flatt, J. P., and E. G. Ball. 1964. Studies on the metabolism of adipose tissue. XV. An evaluation of the major pathways of glucose catabolism as influenced by insulin and epinephrine. *J. Biol. Chem.* **239**: 675.
33. Knopp, R. H., H. J. Ruder, E. Herrera, and N. Freinkel. Carbohydrate metabolism in pregnancy. VII. Insulin tolerance during late pregnancy in the fed and fasted rat. *Acta Endocrinol.* In press.
34. Leake, N. H., and R. L. Burt. 1966. Response of rat adipose tissue to insulin during pregnancy. *Amer. J. Obstet. Gynecol.* **96**: 131.
35. Leake, N. H., and R. L. Burt. 1969. Effect of HPL and pregnancy on glucose uptake in rat adipose tissue. *Amer. J. Obstet. Gynecol.* **103**: 39.
36. Clark, C. M., Jr., G. F. Cahill, Jr., and J. S. Soeldner. 1968. Effects of exogenous insulin on the rate of fatty acid synthesis and glucose C^{14} utilization in the twenty-day rat fetus. *Diabetes.* **17**: 362.
37. Hytten, F. E., and I. Leitch. 1964. The Physiology of Human Pregnancy. Blackwell Scientific Publications Ltd., Oxford. 42.
38. Hytten, F. E., and I. Leitch. 1964. The Physiology of Human Pregnancy. Blackwell Scientific Publications Ltd., Oxford. 50.
39. Grieg, M., M. G. Coyle, W. Cooper, and J. Walker. 1962. Plasma progesterone in mother and foetus in the second half of human pregnancy. *J. Obstet. Gynaecol. Brit. Commun.* **69**: 772.
40. Samaan, N., S. C. C. Yen, H. Friesen, and O. H. Pearson. 1966. Serum placental lactogen levels during pregnancy and in trophoblastic disease. *J. Clin. Endocrinol.* **26**: 1303.
41. Ball, E. G., and R. L. Jungas. 1961. On the action of hormones which accelerate the rate of oxygen consumption and fatty acid release in rat adipose tissue *in vitro*. *Proc. Nat. Acad. Sci. U. S. A.* **47**: 932.