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LIPOGENESIS IN THE GENETICALLY OBESE ZUCKER RAT

by

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ABSTRACT

In situ fatty acid synthesis has been measured with ^3H_2O in lean and obese Zucker (fa/fa) rats. The accumulation of fatty acids was increased in both the liver and adipose tissue of young fa/fa rats as a result of both an increased rate of lipogenesis and an increase in tissue mass. Whereas total hepatic lipogenesis increased with age, total adipose tissue lipogenesis decreased in older fa/fa rats. Experiments with hepatectomized rats showed that the liver was the major site of the excess fatty acid synthesis in the fa/fa rats. The enhanced rate of lipogenesis in fa/fa rats was abolished by either pair feeding or streptozotocin treatment. The results suggest that the increased fatty acid synthesis in fa/fa rats is secondary to the hyperphagia, hyperinsulinaemia and increased mass of hepatic and adipose tissues. Sucrose feeding resulted in an increased hepatic and adipose tissue lipogenesis and in insulin levels in lean animals with an increase in hepatic lipogenesis and insulin levels in the fa/fa rats. Adrenalectomy decreased the rates of lipogenesis in both the liver and adipose tissues, insulin levels and weight gain of fa/fa rats.

Pre-obese fatty rats have been identified by their lower rectal temperature ($34.6 \pm 0.2^{\circ}C$ v $35.4 \pm 0.3^{\circ}C$) from day 16 onwards. Hepatic lipogenesis, hepatic glucose-6-phosphate dehydrogenase, hepatic acetyl-CoA carboxylase and insulin levels remained unchanged in suckling pre-obese rats and increased only after weaning when all the values for the obese were significantly different from values obtained for lean rats. However, adipose tissue lipogenesis, glucose-6-phosphate dehydrogenase, acetyl-CoA-carboxylase and adipocyte size were all significantly increased in the pre-obese suckling rats from day 10 onwards. The results suggest that the primary genetic defect in the fatty rats may be related to either a defective thermogenic process or a defective control of adipose tissue lipogenesis.

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INTRODUCTION

Obesity is a frequent nutritional disturbance in the developed countries. Approximately a third of the adult population in Britain is above the range of weight for height which is associated with greatest life expectancy, increased incidence of diabetes, gastrointestinal disorders and joint deformities. Obesity results from an imbalance in the equation:

Energy intake = Energy expenditure + Energy storage + Heat loss

either due to increased food intake, increased efficiency of utilization leading to increased energy storage or decreased energy expenditure or heat loss.

Reported data shows that obesity is not only prevalent in the adult population but is reported in infants and school going children too. Estimates of obesity during the first year have been made by Skukla et al (1972) who found that 16.7% of 300 babies weighed more than 20% above the expected value and 27.7% weighed between 10-20% more than the expected value. The prevalence of obesity during the school years has been estimated at 2-3% by Howard et al (1971) and increases during puberty especially in girls when it becomes as high as 10-15% (Canning and Mayer, 1966).

Asher (1966) and Eid (1970) have shown that excessive weight gain during the first six months of life is associated with a greater incidence of obesity during the later years of childhood. Studies on twins (Newman et al, 1937, and Bakwin, 1973) suggest that genetic factors are of some importance and that there is an effect of genetic influence on the control of body weight. The effect of overnutrition may be the stimulation of growth and this could be most marked when overnutrition occurs during the first few years of life, when the capacity for accelerating the rate of cell multiplications is the greatest. The size and number of fat cells are increased in adults and older children who were obese by the age of one (Brook et al, 1972).

Obesity is a disease of multiple etiology. The earliest age at which juvenile onset obesity begins is not known nor whether it is primarily due to nutritional excess during infancy or if it starts in utero. Also unknown is whether increased cellularity is usually the result of overnutrition

during the first year of life or that overnutrition at this time always results in an increased rate of cell multiplication. Long term follow up studies are needed to show whether increased cellularity will persist indefinitely if weight is lost and whether the tendency for obesity to recur even after successful weight loss is the result of increased cellularity.

Neither the causes of obesity nor a satisfactory cure for obesity are known. The dietary and non-dietary approaches that have been made in the treatment of obesity either possess high risk factors or poor long-term prospects for the maintenance of weight loss.

Similarly non-dietary approaches like intestinal by-pass surgery (Quaade, 1974) may result in abdominal discomfort, liver dysfunction and arthritis whereas hypothalamic ablation (Quaade, 1974) resulted in no weight loss. The use of anorectic drugs are reported to have side effects (Samuel and Burland, 1974) like addiction to amphetamines. Thyroid hormones have been used as thermogenic drugs but there is a danger of thyrotoxicosis (Samuel and Burland, 1974). However, thyroid hormone therapy can induce weight losses accompanied by an increased sensitivity of fat cells to the lipolytic action of adrenaline in humans.

Since experimental work on humans is very restrictive, one approach to understanding the causes of obesity and the inter-relationships between hereditary and environmental factors is the study of obese animal models (Bray and York, 1971b). These models also allow a detailed study of the extent to which dietary manipulations can influence both the time of manifestation and the severity of a genetically determined metabolic disorder. Many animal models of obesity are known. However, some of these inherit obesity genetically whereas in others obesity is produced either by hypothalamic destruction using either selective electrolytic destruction or chemical agents, or by methods that deal with endocrine or dietary manipulations as reported by Bray and York (1971b). These varying animal models can thus be studied to try and understand the different aspects of human obesity and diabetes depending upon the mode of inheritance of the various metabolic disorders.

Lipogenesis:

Biosynthesis of fatty acids:-

Pathway for fatty acid synthesis
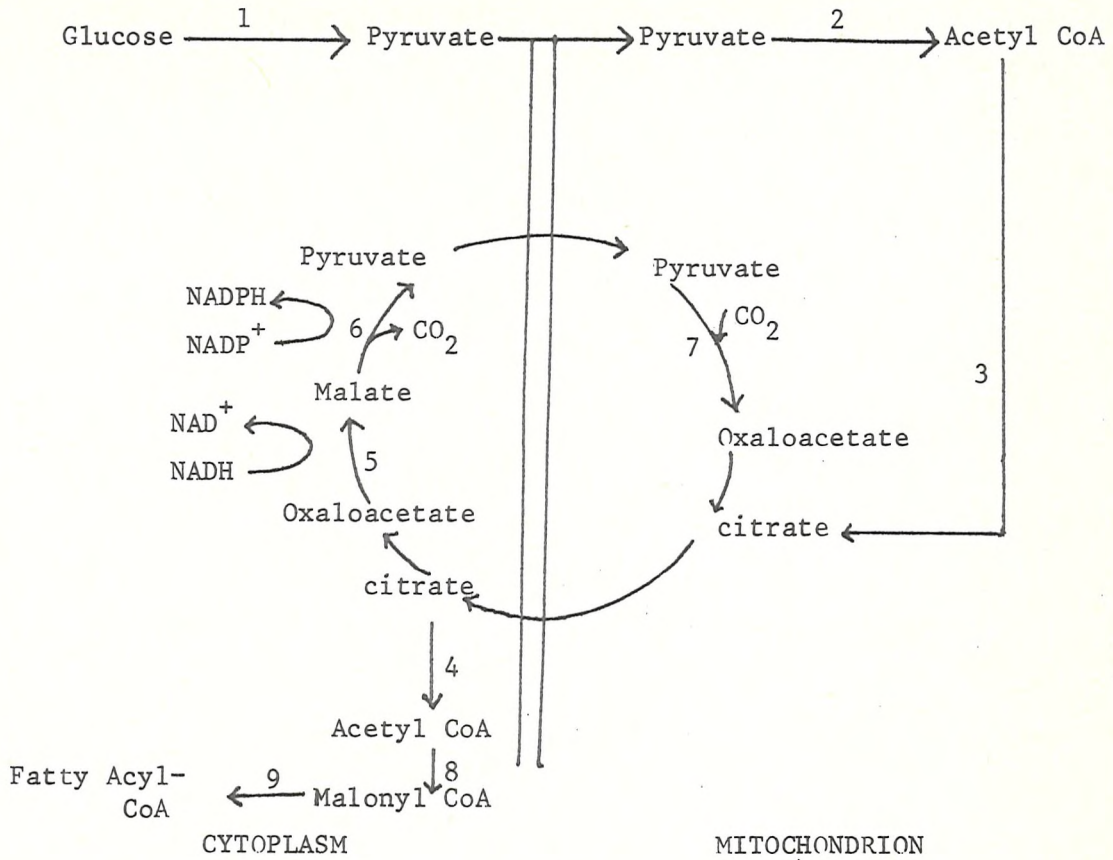
1   Glycolysis
2   Pyruvate dehydrogenase
3   Citrate synthetase
4   ATP - citrate lyase
5   NAD^+ linked malate dehydrogenase
6    $NADP^+$ linked malate dehydrogenase-malic enzyme
7   Pyruvate carboxylase
8   Acetyl CoA carboxylase
9   Fatty acyl CoA synthetase

Fatty acids are synthesized in the cytoplasm from acetyl CoA, a
substrate that can be derived from carboyhydrates by the oxidative decarboxy-
lation of pyruvate or the oxidative degradation of some of the amino acids.
Since acyl CoA derivatives cannot cross the inner mitochondrial membrane
the acetyl group may be transferred to carnitine and exit from the mito-
chondrion as an acyl carnitine. However, evidence indicates that this is
not the major mechanism for the efflux of mitochondrial acetyl CoA and

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instead most of the acetyl CoA leaves the mitochondrion in the form of
citrate. In the cytoplasm citrate is cleared to acetyl CoA and oxalo-
acetate by citrate lyase. The regulatory step in fatty acid synthesis is
the carboxylation of acetyl CoA to malonyl CoA by acetyl CoA carboxylase
since this is the first committed step. However,
acetyl CoA carboxylase and fatty acid synthetase like pyruvate dehydrogenase
can be converted from the phosphorylated inactive form to the dephosphory-
lated active form (Carlson and Kim, 1974a; Carlson and Kim, 1974b;
Qureshi et al, 1975) and so all these enzymes could coordinately control the
rate of fatty acid synthesis. This enzyme is activated by citrate or
isocitrate to the polymeric form (Gregolin, C. et al, 1968). In the next
reaction, catalyzed by the fatty acid synthetase complex, the acetyl and
malonyl groups are condensed to acetoacetyl ACP and finally reduced to
butyryl ACP. Following this series another malonyl ACP condenses with the
butyryl ACP and the sequence is repeated. Fatty acid synthesis usually
stops after the chain is 16 carbon atoms long. Much of the NADPH needed
for the reductions is obtained from the initial reactions of the pentose
phosphate pathway (hexose monophosphate shunt). In the liver these fatty
acids may be oxidized or utilized in the synthesis of triglycerides and/or
phospholipid. Since the liver has a limited capacity for storage of
triglyceride the excess is secreted into the blood in the form of very low
density lipoproteins (VLDL).

Fatty acid synthesis can be controlled by the supply of substrates,
the activity of enzymes controlling fatty acid synthesis or the supply of
the reducing power NADPH. Since fatty acid synthesis can be regulated
by substrate (acetyl CoA) availability factors regulating glycolysis could
be important, for example glucose transport, hexokinase and phosphofructo-
kinase. However, insulin can regulate fatty acid synthesis at the level
of pyruvate dehydrogenase by converting it into the active dephosphorylated
form. Acetyl CoA carboxylase, a regulatory enzyme in the control of fatty
acid synthesis, is activated by citrate or inhibited by long chain fatty
acyl CoA so that an increase in citrate or a decrease in concentration of
fatty acyl CoA could be responsible for the increase in fatty acid synthesis.
A proportion of the reducing power NADPH required for fatty acid synthesis
is provided by the oxidation of glucose in the pentose phosphate pathway so
that this could also regulate fatty acid synthesis.

Effect of sucrose feeding on hepatic and adipose tissue lipogenesis

Pathway of fructose metabolism in the liver

Fructose

ketohexokinase

Fructose-1-phosphate

Aldolase

Dihydroxyacetone phosphate + Glyceraldehyde

Triosephosphate
Isomerase

ATP

Triokinase

ADP

Glyceraldehyde-3-phosphate

Glycolysis

Pyruvate
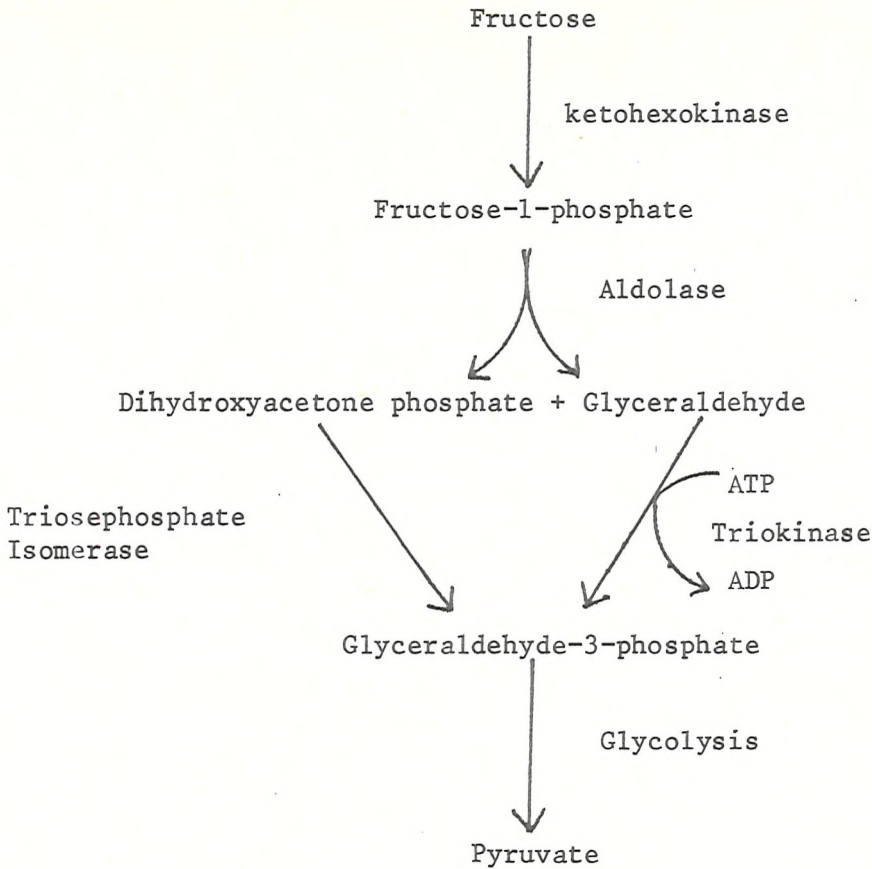
Fructose is phosphorylated in the liver by fructokinase to fructose-1-phosphate. This intermediate is converted by an aldolase to glyceraldehyde and later to glyceraldehyde-3-phosphate by a triokinase. This pathway is very active in the liver (Sillero et al, 1969) and fructose can be phosphorylated in the liver at a much faster rate than glucose (Spiro and Hastings, 1958; Zakim et al, 1967). By this active pathway fructose bypasses the first two slow steps of glycolysis catalyzed by glucokinase and phosphofructokinase, so that

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                    Glucose

                         Hexokinase

        Glucose-6-phosphate
                         Phosphoglucoisomerase
        Fructose-6-phosphate
                         Phosphofructokinase
        Fructose-1-phosphate
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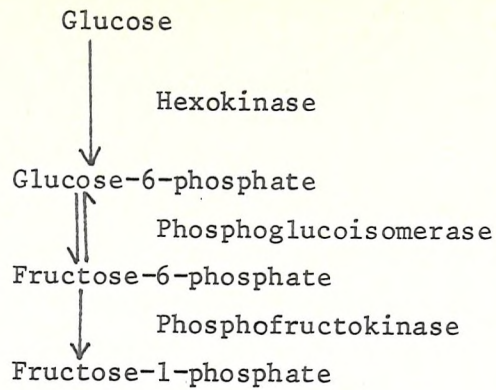
fructose feeding results in 40-70% higher levels of pyruvate, acetyl CoA
and malate in the liver than does glucose feeding (Zakim et al, 1967).
This explains the greater synthesis of fatty acids from   fructose
by liver slices of animals fed fructose compared to glucose. It is possible
that intermediates at the triose phosphate step or beyond, for example,
acetyl CoA, citrate leads to the induction of fatty acid synthesis as well
as other lipogenic enzymes (Takeda et al, 1967).

In the adipose tissue fructokinase is absent (Adelman et al, 1967) and
so fructose must be phosphorylated to fructose-6-phosphate by hexokinase.
Hexokinase has a twenty fold higher affinity for glucose than fructose and
therefore stimulatory intermediates would be lower in concentration in adi-
pose tissue upon feeding of fructose rather than glucose.

The fatty rat

The cellularity characteristics of the Zucker fatty rat make it a good
model to study obesity in humans of the juvenile onset type (Zucker and
Zucker, 1961; Zucker and Zucker, 1962; Zucker and Zucker, 1963; Johnson
et al, 1971).

Zucker and Zucker (1961) described this rat when it appeared as a
spontaneous mutation in a cross between the Sherman and Merck Stock M rats
(Zucker and Zucker, 1963). The fatty rat inherits obesity as an autosomal
Mendelian recessive trait. The one gene one enzyme concept was formulated
by Beadle and Tatum. So that a single nucleotide in the genetic code
for some peptide has been deleted or replaced by another nucleotide
The abnormal DNA sequence is transcribed into an abnormal m.RNA and in turn
a defective peptide results during translation of the m.RNA. The fatty
rat being sterile is bred from heterozygote lean parents.

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                    ♂                    ♀
              Fa fa              Fa fa          lean heterozygote parents
                       \         /
                        \       /
    Fa  Fa          Fa fa   Fa fa         fa fa
homozygote lean     heterozygote lean     homozygote obese
```
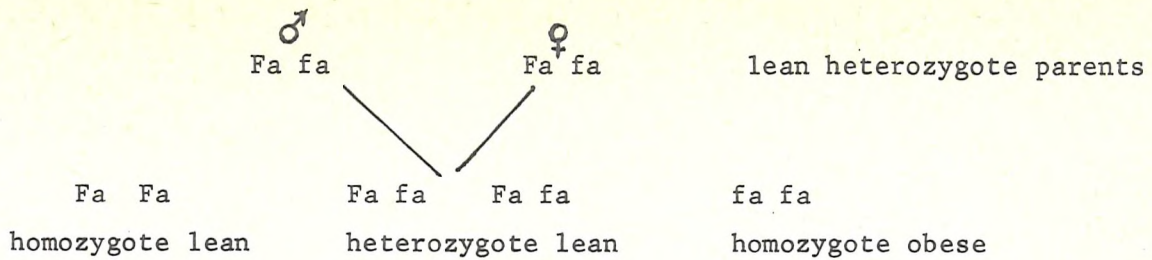
The three genotypes employed are the Fafa and FaFa both of which are
lean being normal in body weight and composition and at present phenotypically
indistinguishable from each other.   The third is the homozygous recessive
fafa individual described as the fatty rat being visually indistinguishable
from its lean littermates until 4-5 weeks of age, after which it becomes
excessively obese.

Characteristics of the fatty rat

Some of the characteristics of the fatty rat are hyperphagia, hyper-
insulinemia, hyperlipogenesis, hyperlipemia, hypertrophy and hyperplasia
of the adipocytes, hypothermia and changes in the endocrine system and some
of the enzymatic patterns (Bray and York, 1971b; Herberg and Coleman, 1977).
However, the major problem related to these reported characteristics is the
differentiation between secondary and primary changes owing to the diffi-
culties arising from the inability to identify the fatty rats before they
can be visually detected at 4-5 weeks by which time most of these character-
istics are already present.

Recent studies have now reported some of these characteristics to appear
in the fatty rat prior to weaning whereas others appear only after weaning.

Characteristics of the preweaned (pre-obese) fatty rats

Adipocyte cellularity as reported by Greenwood and Hirsch (1974) in
normal rats shows that the cell number can increase from birth to 12-14 weeks
and after maturity no change in number occurs and only the adipocyte size
varies due to lipid filling.   Thus once established the cell number is not
normally altered by post weaning manipulation like starvation or experi-
mentally produced hyperphagia (Hirsch and Han, 1969) although feeding high
fat diets may cause adipocyte hyperplasia (Lemmonier and Alexiu, 1974).
However, preweaning nutritional changes like rearing the animals in large

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litters before weaning have been reported to reduce the number of adipocytes seen in the adult rat (Knittle and Hirsch, 1968). The fatty rats differ from the Sprague Dawley in that the adipocyte number as well as size continues to increase beyond week 14 as in normal rats with the result that the adult animal has approximately double the cell number (Johnson et al, 1971). The adipose cell number in the preweaning fatty rats is predominantly controlled by the genotype rather than early nutrition. Johnson et al (1973) have shown that whereas over feeding can result in an increase in cell number in both lean and obese preweaned animals, undernutrition only affected the cell number of the lean animals with no effect on the pre-obese.

Boulange et al (1978) used the method of cell sizing to identify the pre-obese pups from lean littermates. They found that fat cells in biopsy samples of inguinal subcutaneous adipose tissue were always larger in the pre-obese rats than in controls as early as 5-7 days of age so that in the obese rats the fat pads were constantly heavier and the cell size larger. The cell number was slightly higher than in the controls at 1-2 weeks but after that the further increase was at a slower rate (1.8 fold during the 3rd and 4th week in the obese) compared to the controls (3 fold increase in the 3rd week and 2 fold increase in the 4th week) with the result that there were less cells in the obese at 4 weeks. This observation is similar to that reported by Johnson et al (1971) who observed a tendency in 3 week old obese rats to have fewer fat cells than controls possibly due to a delay in new fat cell proliferation during the onset of obesity.

Kaplan (1977) identified the pre-obese rats with 99% accuracy by measuring oxygen consumption. As early as 15-17 days the pre-obese exhibited low oxygen consumption. At around the same age we have shown that rectal hypothermia can be used to identify the pre-obese rats from lean littermates (Godbole et al, 1978).

Enzymes associated with proliferative activity like adipose tissue thymidine kinase and DNA polymerase show a marked increase in activity in the adipose tissue of the pre-obese rats. Lipoprotein lipase activity is also increased in the adipose tissue of the pre-obese rats (Boulange et al, 1978b) reflecting an increased storage capacity of circulating triglycerides by the adipose tissue.

An increase in the percentage body lipid has been observed (Johnson et al, 1971; Bell and Stern, 1977) in the pre-obese rats from two weeks. This

could result from either an increase in food intake, an increase in lipo-protein lipase activity in the adipose tissue or an increase in situ tissue lipogenesis or a decrease in energy expenditure. There are very few studies as yet on these differing possibilities. Turnover of ^3H_2O has been used to estimate milk intake of suckling pre-obese fatty rats but no differences were detectable between lean and pre-obese pups. Similarly no differences in the activity of lean and pre-obese pups have been reported. Studies on adipose tissue and hepatic lipogenesis in pre-obese fatty rats are reported in this thesis.

Hyperinsulinaemia is a characteristic of all forms of obesity other than that resulting from feeding high fat diets. It is possible therefore that hypersecretion of insulin could be the primary cause of hyperlipogenesis and increased food intake. However, serum immunoreactive insulin (IRI) levels are normal in two week old pre-obese fatty rats but may be slightly increased by three weeks (Zucker and Antoniades, 1972; Godbole et al, 1978).

From the reported studies it appears that changes in body fat content precede increased insulin levels, increased food intake and decreased activity in the pre-obese rats. However, hypometabolism as evidenced by decreased oxygen consumption and decreased rectal temperature is observed at a similar time to the initial increase in body fat. The primary defect which could possibly be linked with either lipogenesis or hypometabolism awaits identification.

Characteristics of the weaned fatty rat

Hyperphagia

Food intake measurements show that food intake increases in the obese mutants soon after weaning compared to their lean littermates. The hypo-thalamus has been implicated to play a fundamental role in the regulation of food intake, by two opposing mechanisms, one that is located in the ventromedial area of the hypothalamus that brings about satiation of appetite (satiety centre) while the ventrolateral area initiates feeding (feeder centre) (Anand, 1961) so that the ventromedial area exerts an inhibitory influence upon the ventrolateral hypothalamus thus modulating the food intake. The actual mechanism of activation of this satiety centre is not known, however, rate of glucose utilization as sensed by proposed glucoreceptors

in the ventromedial nuclei (Mayer, 1955), regulation associated with body
fat level (Kennedy, 1966) and regulation by body temperature changes
(Brobeck, 1960) have all been designated as types of food intake regulatory
mechanisms.

The reported experimental lesions of the hypothalamus resulting in
hyperphagia and finally obesity have been shown more recently to result from
damage to the paraventricular nucleus or to nerve fibres running past to
the ventromedial hypothalamus rather than the ventromedial hypothalamus
itself. Similar conclusions were reached from experiments utilising either
knife cuts (Becker and Kissileff, 1974) or the local or systemic administra-
tion of gold thioglucose (Debons et al, 1974). Damage to this satiety
region results in hyperphagia and finally obesity similar in extent to that
observed in genetically obese animals. However, the regulation of food
intake in the two models differs indicating that the hyperphagia in the
obese fatty rats does not reflect a simple lesion of comparable location
and quality to that observed in rats with hypothalamic injury (Bray and
York, 1972; Greenwood et al, 1974). The hypothalamically lesioned animals
are more influenced by sensory properties of their food. The genetically
obese fatty rat behaves more like the normal lean animal compared to the
hypothalamically obese rat. The fatty rat shows an increase in food
intake to compensate for food dilution with cellulose and a decrease in
food intake to compensate for a calorically dense 60% fat diet (Bray and
York, 1972). Also the obese fatty rats are not finicky eaters, e.g., their
food intake is not affected by quinine adulteration. Greenwood et al (1974)
have shown that the fatty rat will increase the frequency of bar pressing
in order to maintain pellet rewards at a much higher ratio of bar presses
to pellet reward than will animals with hypothalamic obesity. The fatty
rats unlike the normal lean controls show a loss of the normal diurnal
variation in meal pattern (Wangsness et al, 1978; Martin et al, 1978).

It is thought that a defect in protein deposition in the fatty rat
could play a role in the regulation of food intake in the obese rat. The
rate of protein deposition is much lower in the obese rats when given com-
parable amounts of food to lean (Radcliffe and Webster, 1976) but is normal
when allowed food ad libitum. However in this situation the percentage of
total energy stored that was retained in protein was only 14% in the obese
compared to 75% in lean rats (Puller and Webster, 1974). Since the obese

rats use food less efficiently to promote protein deposition it is possible that they eat more to maintain normal rates of protein deposition. In the fatty rats food intake and rate of lipid deposition decreased with increasing the dietary protein content (Radcliffe and Webster, 1976; Jenkins and Hershberger, 1978). Compared to food intake on a high carbohydrate or high fat diet the intake of obese rats fed the high protein diet was decreased to a greater extent than for the lean rats, so that the fatty rats lost weight but continued to deposit excess amounts of body fat.

Results of behavioural studies indicate a relationship between feeding behaviour and levels of brain catecholamines, of which norepinephrine and dopamine have both been implicated. Norepinephrine but not dopamine concentration in the median eminence shows twice the concentration in the obese compared to the lean littermates (Cruce et al, 1976) and decreased concentrations in the paraventricular nucleus (Cruce et al, 1976).

The mechanisms underlying the hyperphagia in the obese rat are not understood, however, it appears that neural endocrine mechanisms could possibly be responsible for biochemical changes underlying the defects in hypothalamic regulation of feeding in the obese rats.

Pancreatic Function

Insulin

Increased insulin levels have been reported in these Zucker obese animals from as early as three weeks of age (Zucker and Antoniades, 1972) and this appears to be a major defect of both spontaneous or experimentally produced animal obesities. Restriction of food intake by pair feeding lowers insulin levels; however, the extent to which insulin levels are reduced vary from study to study depending on the age of the animals and the duration of pair feeding. Pair feeding reduced insulin levels but not to normal values (Lemonnier et al, 1974; Stern et al, 1972; York and Bray, 1973) whereas pair feeding of weanling obese rats on a meal feeding regime straight after weaning restored insulin levels to lean values (Godbole and York, 1978). In yet another study only restriction of dietary intake to threequarters of that eaten by lean animals (York and Bray, 1973) or total starvation reduced insulin levels to values of the lean (Zucker and Antoniades, 1972).

This increased secretion of insulin in the fatty rat is associated
with changes in pancreatic histology. The precise time when these changes
occur is not known.   An increase in size and the number of pancreatic
islets in fatty rats and rats with hypothalamic obesity is reported (York
et al, 1972b) and this is accompanied with increased islet diameters. a
highly developed Golgi appratus and endoplasmic reticulum, increased
vascularisation and degranulation of the islets by the 8th week of life
in the fatty rat (Shino et al, 1973).   Insulin concentration is increased
in the pancreas of the obese rats (Lemonnier et al, 1974) and the release
of insulin from isolated islets in response to various secretagogues in
vitro and in situ is abnormal, so that the pancreatic islets from obese
rats shows hypersecretion of insulin to glucose (Stern et al, 1972; Schade
and Eaton, 1975) but a normal suppression of glucagon secretion in response
to glucose (Eaton et al, 1976; Eaton et al, 1976b). However, the biological
potency and the structure of insulin of the fatty rat are both normal
(Laburthe et al, 1975) thus it is unlikely that hyperinsulinaemia could
result from the secretion of a defective insulin molecule.

The initial effect of this hyperinsulinaemia on obesity is possibly
the overstimulation of all target tissues of the hormone (Fohman et al,
1972) which then develop some degree of insulin resistance. Thus the
increased insulin levels are accompanied with a normal (York et al, 1972b)
or slightly elevated serum glucose levels (Zucker and Antoniades, 1972;
Martin et al, 1978; Bryce et al, 1977). The adipose tissue but not the
liver or muscle during the static phase of obesity shows a loss of insulin
sensitivity (Zucker and Antoniades, 1972; York and Bray, 1973; Stern et
al, 1972; Stern et al, 1975). This insulin resistance of the adipose
tissue results in decreased lipogenesis whereas hepatic lipogenesis con-
tinues to increase with age (Godbole and York, 1978). However, the obesity
and hyperinsulinaemia of obese rats does not result in a decrease in
insulin receptor number on hepatic plasma membranes (Broer et al, 1974).
Pancreatic polypeptide concentration is normal in young obese rats but
decreases with age.   Reduction of body weight restores pancreatic poly-
peptide concentrations to lean values (Larsson, 1977).

The increased liver and adipose tissue lipogenesis observed in the
fatty rats along with increased insulin levels could be explained in part
by the metabolic effects of insulin on the liver and adipose tissue and
on some lipogenic enzymes.

Metablic effect of insulin on the adipose tissue

Hyperinsulinaemia can augment lipogenesis from various substrates. The activity of several lipogenic enzymes is increased like pyruvate dehydrogenase in the active unphosphorylated form, an enzyme that liberates acetyl CoA (Mukherjee and Jungas, 1975; Stansbie et al, 1976; Coore et al, 1971; Weiss et al, 1971). Insulin might activate the phosphatase by increasing the intramitochondrial Ca^{++} concentration (Severson et al, 1974). Insulin thus increases the conversion of glucose and glycolytic residue into fat, e.g., pyruvate and lactate. Hyperinsulinaemia may also increase lipoprotein lipase activity with the result an increased uptake of plasma triglycerides may occur (Rath et al, 1974). In earlier studies the primary location of glycerokinase was demonstrated in the liver and kidney and was not found in the adipose tissue of normal rats (Wieland and Suyter, 1957). However, it has now been shown to be present in extremely low concentrations under normal conditions but may increase with obesity (Koschinsky et al, 1971). The decrease in serum insulin following streptozotocin resulted in a decrease in glycerokinase whereas exogeneous insulin increased the enzyme activity pointing to the role that insulin plays in the regulation of glycerokinase in the adipose tissue. Glycerokinase increases the esterification of the newly synthesised free fatty acids or those derived from the very low density lipoproteins (VLDL). Insulin also increases the rate of the pentose phosphate pathway which is responsible for the generation of at least part of the reducing power (NADPH) necessary for fatty acid synthesis.

Insulin by its action on the lipogenic and glycolytic enzymes increases the rate of esterification in the adipose tissue thus resulting in an increased triglyceride deposition in the central vacuole of the fat cells resulting in an increase in adipocyte size. The changes that occur in the adipocyte of the obese rat are represented in the figure by Assimacopoulas J. and Jeanrenaud, B., 1976).
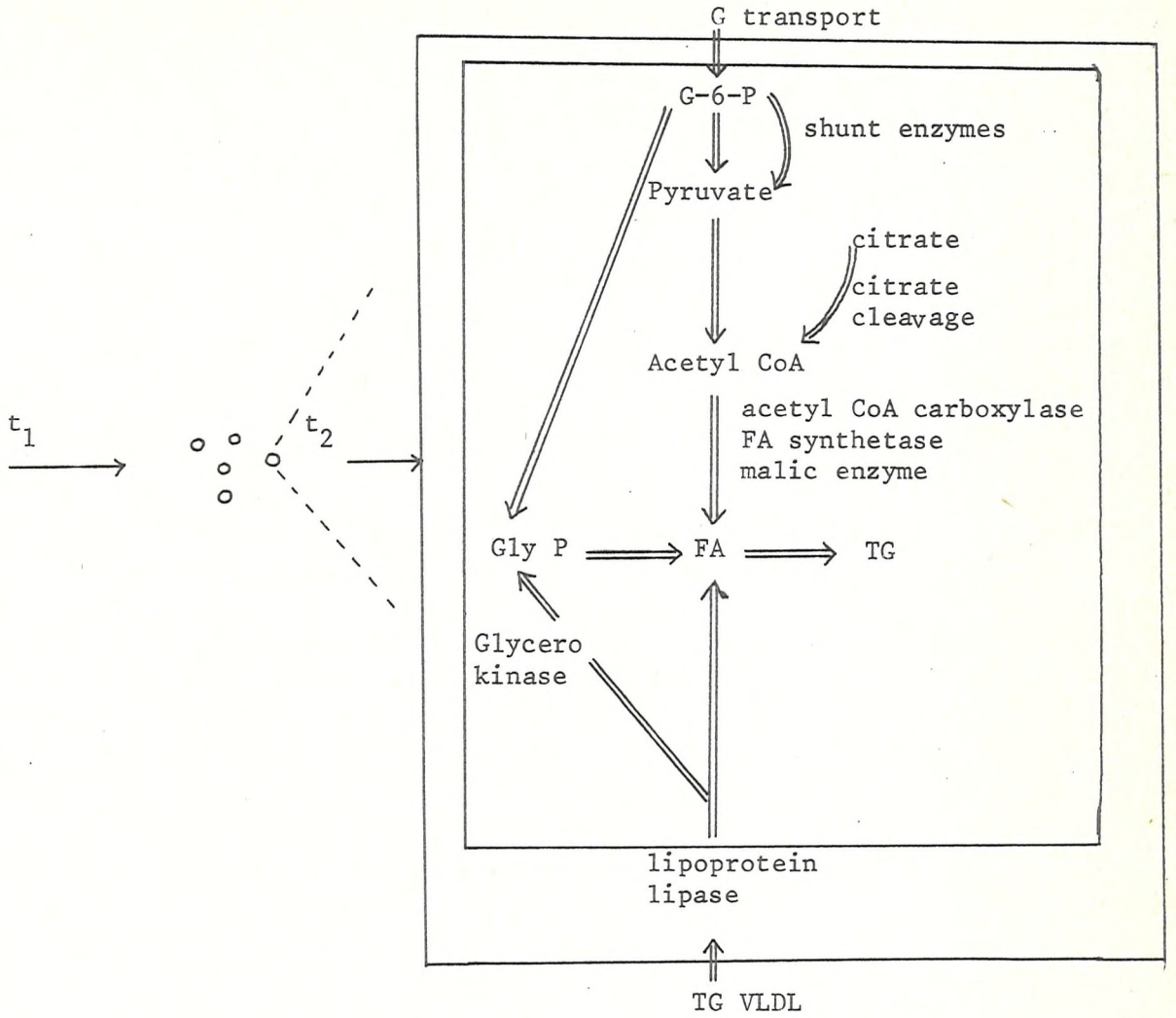
increased cell number

$\Longrightarrow$ pathways increased.

   G. – glucose

 G6P – glucose-6-phosphate

 PDH – pyruvate dehydrogenase

Gly P – glycerophosphate

  FA – fatty acids

  TG – triglycerides

VLDL – very low density lipoproteins

  t_1 – very early changes in obesity that lead to increased fat cell number

  t_2 – early changes due mainly to hyperinsulinaemia that produce the anomalies indicated with resulting increased adipocyte size

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Metabolic effect of insulin on the liver

The liver differs from the adipose tissue in that the transport of glucose across the liver cell membrane is an equilibrium process and is thus not modified by insulin. However, insulin increases lipogenesis from various substrates. Insulin treated rats had an increased hepatic activity of lipogenic enzymes like citrate cleavage enzyme, malic enzyme and pyruvate kinase (McCormick et al, 1978). Similarly insulin administration to diabetic animals increases the hepatic fatty acid synthetase (Lakshmanan et al, 1972) and acetyl CoA carboxylase (Nakanishi, . and Numa S. , 1970) to normal levels. Lakshmanan et al (1972) have shown a twenty fold increase in the actual amount of purified fatty acid synthetase isolated from diabetic rats treated with insulin compared to untreated rats showing that insulin brings about an adaptive increase in the rate of synthesis of the enzyme and not merely an activation of existing enzyme. Similarly, insulin also increases the amount of acetyl CoA carboxylase (Volpe, . and Vagelos, . 1973). Insulin also increases the esterification of fatty acids to triglycerides partly due to an increase in glycerokinase activity whereas oxidation of fatty acids to ketone bodies is decreased. These changes in the obese rats lead to an increase in triglyceride synthesis with a resulting intracellular accumulation of lipids and an increase in the secretion of triglycerides as very low density lipoproteins.

Although insulin cannot modify the transport of glucose across the liver cell membrane it can modify the rates of release and uptake of glucose by the liver both in vivo and in vitro. Besides affecting fatty acid and triglyceride synthesis insulin can regulate glucokinase activity, glycogen synthesis and gluconeogenesis. The amount of glucokinase in the liver appears to be regulated by the plasma levels of glucose and insulin and it has been suggested that insulin causes a specific increase in the synthesis of this enzyme in the liver. These changes in enzyme activity may be responsible in part for the effects of insulin on glucose uptake and release by the liver. Insulin increases the rate of glycogen synthesis in the liver and this may be important in causing an increased glucose uptake by the liver. Gluconeogenesis is decreased in the liver by insulin possibly through the reduced concentration of cyclic AMP.

Glucagon

Circulating glucagon levels have been reported to be decreased (Eaton et al, 1976) and these decrease further with fasting in the obese rat unlike in the lean rats, where glucagon levels increase with fasting (Eaton et al, 1976b). However, the pancreatic glucagon concentration does not appear to be significantly changed in the obese rats (Laburthe et al, 1975; Lemmonier et al, 1974; Larsson et al, 1977) and the physiologic suppression of glucagon release by glucose administration remains intact (Eaton et al, 1976b). The reduced secretion of glucagon could be a consequence of the elevated concentration of insulin, free fatty acids and glucose (Eaton et al, 1976b) for insulin has been reported to suppress glucagon secretion in vitro in isolated pancreatic islets (Backman and Mawhinney, 1973). Since the fatty rats are characterised by an increased insulin secretion and a reduced secretion of glucagon this results in an increase in the circulating insulin: glucagon ratio and this could cause in part the hyperlipemic condition of the obese rat for glucagon is an effective lipid lowering hormone in all species, (Eaton,   ., 1973b; Paloyan and Harper, 1961). Glucagon binding to hepatic plasma membranes is also normal in the obese rat (Broer et al, 1974). Glucagon binding appears to be more dependent on the dietary status of both animals rather than the extent of obesity. There is no difference in immunological or biological activity of glucagon isolated from the pancreas of lean and obese Zucker rats (Laburthe et al, 1975).

Glucagon stimulates triglyceride lipase activity in the adipose tissue and this results in fatty acid mobilization. In the liver glucagon raises the intracellular concentration of cyclic AMP thus stimulating glycogenolysis and gluconeogenesis. Glucagon reduces the induction of fatty acid synthetase, acetyl CoA carboxylase (Volpe and Marasa, 1975) and glucose-6-phosphate dehydrogenase   activities upon refeeding starved rats. An intravenous injection of glucagon results in a 55 to 70% decrease in acetyl CoA carboxylase activity (Klain,   . and Weiser,   ., 1973).

Growth hormone and prolactin

Prolactin levels are two to five times higher in the lean compared to the obese rats (Martin et al, 1978; Martin,   . and Gahagan,   ., 1977). Similarly growth hormone levels are severely depressed in obese rats with

greatest difference between lean and obese rats observed during the light periods (Martin et al, 1978; Martin and Gahagan, ., 1977). These differences in growth hormone secretion may be responsible for the impaired nitrogen retention in the obese rats. Even pair feeding of obese rats from weaning to 14 weeks of age showed decreased levels of serum growth hormone and prolactin (Martin, ., and Gahagan, ., 1977) showing that hyperphagia is not essential for suppression in serum growth hormone and prolactin observed in the genetically obese rats. However, the role of both these in the development of obesity is not understood.

Adrenal function

Corticosterone levels are highest at the beginning of the dark cycle and decrease drastically thereafter in the lean rats but the obese rats show no distinct corticosterone secretion pattern (Martin, . et al, 1978). However, Yukimura et al (1978) have shown no differences in corticosterone secretion pattern in lean and obese animals. Increased pituitary stimulation could be responsible for the adrenal corticosteroid hypersecretion because hypophysectomy prevents weight gain in the obese rat (Powley, . and Morton, ., 1976). The impaired insulin secretion associated with lower plasma insulin concentrations in adrenalectomised rats Yukimura et al, 1978) and conversely the increase in plasma insulin levels on glucocorticoid administration (Kirk et al, 1976) suggests that hypersecretion of adrenal steroids could play an important role in the hypersecretion of insulin in the obese rats.

Administration of glucocorticoids to adult lean rats reduces the activities of fatty acid synthetase and acetyl-CoA carboxylase in the adipose tissue with no change in the liver whereas adrenalectomy produces an increase in activities of these lipogenic enzymes in the adipose tissue with no change in the liver (Volpe, . and Marasa, ., 1975).

Thyroid function

The concentration of thyrotropin, the uptake of radioactive iodine and circulating concentrations of thyroid hormones are diminished in the obese rat, suggesting that it may be hypothyroid (Bray, . and York, ., 1971; York, . et al, 1972). This idea is supported by the lower metabolic

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rate of the obese rat compared to its lean littermates (Bray, ., 1969).
The hypothyroid state however cannot be prevented by food restriction
(Bray et al, 1973).   The thyroid stimulating hormone concentration of the
pituitaries is reported to be normal in the obese rat (Bray, ., York,
1971) and thyroid releasing hormone injection produced a normal increase
in circulating thyroid stimulating hormone while propylthiouracil treatment
was ineffective (Bray, ., York, 1971) so that an impairment in the control
of thyroid stimulating hormone has been postulated as a basis for the
hypothyroidism of the obese rat.   Recent observations that after adrenalectomy of
obese rats [131]I uptake by the thyroid returns to lean control levels (Yukimura
et al, 1978) suggests that the observed thyroid hypofunction in the obese
rats may be secondary to the hypersecretion of adrenal corticosteroids.

Reproductive function

     The female obese rat is infertile and accompanied by a delay in vaginal
opening, prolonged estrus and lack of estrus cycles (Saiduddin, . et al,
1973), reduced uterine and ovarian weights which cannot be corrected by
restricting food intake to that of lean littermates (Bray, . et al, 1973).
All these changes are consistent with low circulating levels of oestrogen.
However, follicle stimulating hormone and leutinizing hormone levels are
normal (Bray, . et al, 1973).   When obese and lean rats were treated with
estradiol 17β, the uterine weight of castrated lean and obese rats
increased with increasing dose of estradiol thus suggesting that the small
uterus in the obese rat may be due to decreased estrogen levels (Bray, .
et al, 1976).

     The male obese rats are also abnormal and have a decreased testicular
size (Deb., and Martin, ., 1975).

Lipogenesis in the obese rat

     Hepatic lipogenesis increases soon after weaning in the obese rats
and it remains higher than in the lean rats at all times.   The liver is
the main site for the increased lipogenesis in the obese rats and this
has been shown by hepatocyte preparations (Bloxham, . et al, 1977) as

well as by in vivo experiments (Martin, ., 1974; Triscari, . and
Sullivan, ., 1977; Godbole, . and York, , 1978). The total increase
in hepatic lipogenesis in the obese rats occurs not only due to increased
rates of lipogenesis but also due to the increase in tissue weights with
age. The increased hepatic lipogenesis results in an increased secretion
of very low density lipoproteins (Schonfeld, . et al, 1974; Schonfeld, .
and Pfleger, ., 1971) and serum hypertriglyceridimia (Barry and Bray, .,
1969). The occurrence of hypertriglyceridimia in the obese rat in spite
of an increased adipose tissue lipoprotein lipase activity (Gasquet et
al, 1973) suggests an imbalance between secretion and removal of triacyl-
glycerols from the blood. However, when fatty rats were put on a restricted
diet of 12g/d for 2 months starting from when they were 3 months they showed
a very faint or absent pre β (very low density lipoprotein) band compared to
ad libitum fed obese rats (Koletsky, . and Puterman, ., 1976).

The fatty acids used for glyceride synthesis could come either from
endogenous synthesis in the liver or from the circulation. Studies have
shown that the liver of the obese is not able to utilise glucose as a
carbon source for lipogenesis (Bloxham, . et al, 1977; Clark, . et al,
1974) whereas the hepatocytes of the obese rats show an increased incorpora-
tion of pyruvate and lactate carbon as substrates for fatty acid synthesis
(Bloxham, . et al, 1977).

Since lipogenesis is very sensitive to both increased food intake and
insulin levels many studies have been reported to try and identify the
factors responsible for the hyperlipogenesis of obese rats. Studies on
pair feeding of obese rats show that the obese rats still continue to
accumulate excessive fat compared to their lean littermates in spite of
their similar food intakes (Bray, . et al, 1973; Zucker, , 1975).
The incorporation of ^{14}C acetate into fatty acids is reported to be increased
in the pair fed obese rats and this is accompanied by elevated levels of
enzymes associated with lipogenesis (Martin, ., 1974).

Adipose tissue lipogenesis in the obese rat

Fat in the form of triglyceride is the major storage form of energy
and of the three nutrients only fat can be stored in large quantities.
Adipocytes are solely devoted to the function of storing and synthesizing

fat. Studies in rats indicate that the number of adipose cells is determined by early nutrition because overfeeding of weanling rats results in excessive numbers of adipocytes. Obesity is the overall result of increased number of adipocytes as well as an increase in the size of the adipocytes due to the packing with triglyceride. A primary metabolic drive in the body at times of abundant food supply is the conversion of excess calories from carbohydrate into fat so that they can be kept in reserve in the adipose storage depots.

Triglyceride accumulation in adipocytes occurs from fatty acids synthesised within the adipocyte from glucose or those derived through the blood in the form of triglycerides contained in either chylomicrons or very low density lipoproteins. These lipoprotein triglycerides must be hydrolyzed by lipoprotein lipase so that their fatty acid content can enter into the adipose cell. Fatty acid mobilisation from the adipose tissue occurs by the hydrolysis of triglycerides by triglyceride lipase and the fatty acids are released into the blood and transported as a complex with albumin.

In vitro and in vivo studies on the adipose tissue indicate that the rate of adipose tissue lipogenesis is higher in the young obese rats compared to lean littermates but in older animals this lipogenesis decreases in the obese rats to levels below those of lean rats (Bray, ., 1968; Martin, . and Lamprey, ., 1975; York, . and Bray, ., 1973; Godbole, . and York, ., 1978). This decreased rate of lipogenesis reflects the increasing insensitivity of the adipose tissue to the high circulating levels of insulin. Fatty acid esterification is also higher in the obese rats probably due to an adequate supply of α-glycerophosphate in the presence of an increased supply of fatty acids in the fatty rat (Bray, . et al, 1970).

Lipogenic enzymes like 6-phosphogluconate dehydrogenase, acetyl-CoA carboxylase, ATP citrate lyase and malic enzyme of the adipose tissue of the 8 week old fatty rats all show an increased activity compared to lean littermates (Taketomi, . et al, 1975).

Pair feeding of obese rats decreases the rates of adipose tissue lipogenesis as well as glucose-6-phosphate dehydrogenase, malic enzyme and citrate cleavage enzyme activities to those of lean rats (Martin, ., 1974; Martin, . and Lamprey, ., 1975; Godbole, . and York, ., 1978).

The aim of this work on the obese rat was:
(1) to measure lipogenesis in situ in the obese rat;
(2) to investigate the major site of fatty acid synthesis;
(3) the control of fatty acid synthesis;
(4) the primary defect by studying early changes in the suckling rats.

SECTION 2

METHODS

(a) CHEMICALS

The majority of chemicals used in the experiments reported here were of Reagent grade and were obtained from British Drug Houses, Ltd., Poole, Dorset.

Where special chemicals were purchased these are indicated in the text.

(a)ii __ANIMALS__: Obese rats were bred from lean heterozygotes in the departmental animal house. Rats were housed at $22^{o}C$ with a constant light dark cycle (0730-1930).

(b) COMPOSITION OF DIETS USED

All rats were fed either the ordinary rat chow PRD for laboratory rats purchased from Rank Hovis McDougal:

Fat	3%
Protein	20%
Carbohydrate	55%
Vitamins	
Minerals	22%
Fibre	

or in a few experiments they were fed a high sucrose diet:

Sucrose	67%
Casein	23%
Minerals	8%
Vitamins	2%

(c) FATTY ACID SYNTHESIS

The method of Lowenstein (1971) utilizing the incorporation of $^{3}H_{2}O$ into fatty acids was followed. By this method the label is incorporated into the fatty acid at the two NADPH (nicotinamide adenine dinucleotide phosphate - reduced form) dependent reduction steps. However one of the labelled protons is lost at the dehydration step with the final result that for every acetyl unit one tritiated proton gets incorporated.

However exchange of ^{3}H with NADPH increases this above the theoretical.

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$$CH_3 - C(=O) - CH_2 - \overset{|}{\underset{|}{C}}(=O)\text{-S-ACP} \xrightarrow{\;NADPH^* + {}^3H^+ \to NADP^+\;} CH_3 - \overset{|}{\underset{|}{C}}(H^*)(OH^3) - CH_2 - C(=O)\text{-S-ACP}$$

$$\downarrow {}^3H_2O$$

$$CH_3 - CH_2^* - \overset{|}{\underset{|}{C}}(H)({}^3H) - C(=O)\text{-S-ACP} \xleftarrow{\;NADP^+ \leftarrow NADPH^* + {}^3H^+\;} CH_3 - CH^* = CH - C(=O)\text{-S-ACP}$$

Fatty Acids ←
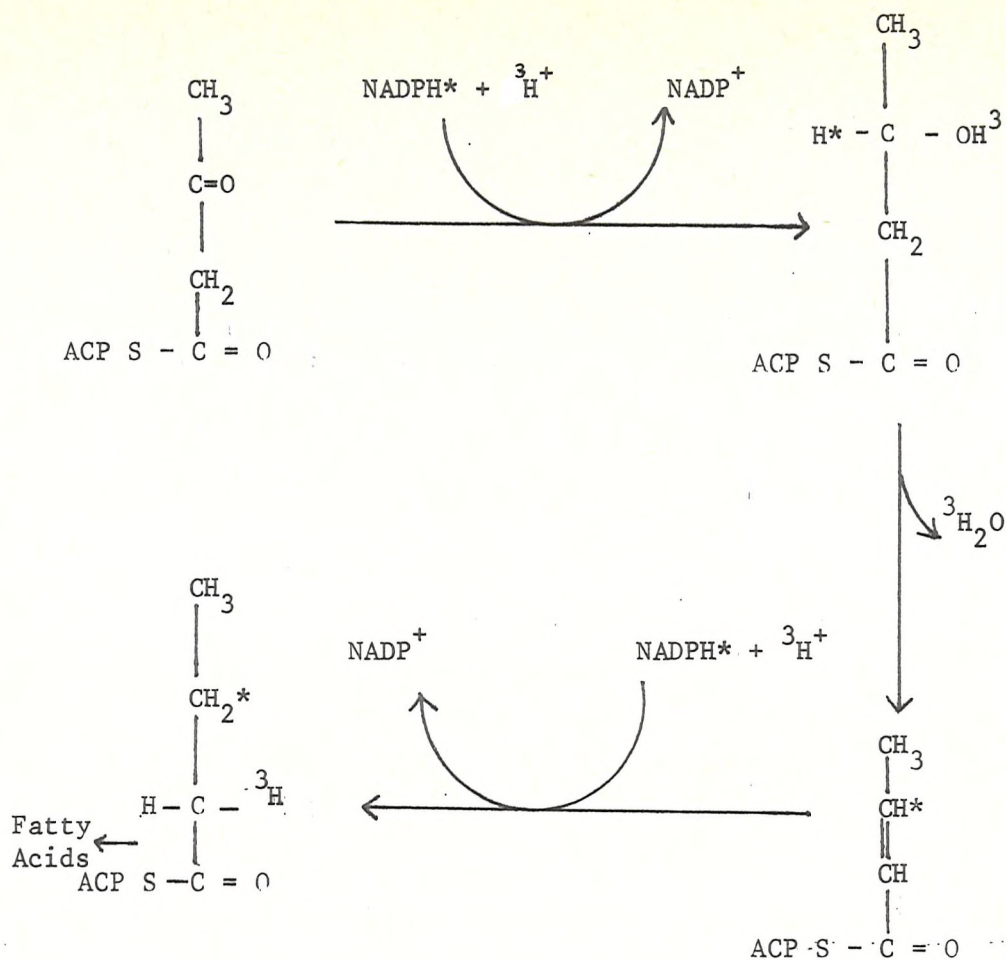
This method for assay of lipogenesis is preferable to the use of [14]C labelled substrates because of the problems that might result from differing pool sizes between different groups of animals and variations of pool size during the course of an experiment.

Rats were anaesthetised with 60 mg/kg nembutal and kept anaesthetised throughout the experimental period or in some experiments unanaesthetised animals were used, both these methods produced similar results. 4mCi ^3H_2O (Radiochemical Centre, Amersham, England) in 9g/ℓ saline was injected into the tail vein. After an hour a blood sample was obtained by cardiac puncture for estimation of serum ^3H_2O specific activity and for serum insulin levels. The liver, the perimetrial and the hind subcutaneous fat pads were dissected free, weighed and then homogenised (1g in 20 ml) in chloroform:methanol (2:1 v/v) for the extraction of lipids (Folch et al, 1957).

This was divided into two aliquots, one aliquot was evaporated to

dryness and then counted whereas the other was evaporated to dryness and
then saponified for 1 h at 85°C in an excess of alcoholic KOH (1 mole/ℓ
KOH in 95% (v/v) methanol). After saponification 4 mℓ of distilled water
was added and the non saponifiable lipids extracted with three washes of
5 mℓ petroleum ether:ether (1:1 v/v). The aqueous phase was then acidified
and the fatty acids extracted with three 5 mℓ washes of petroleum ether:
ether (1:1 v/v).   All fractions were evaporated to dryness in the presence
of benzene:methanol (1:1 v/v) and the radioactivity measured in a Philipps
scintillation counter (efficiency of %^{3}H – 51%) after addition of 10 mℓ of
Tritoscint scintillation fluid (Xylene, 2ℓ; Synperonic NXP 1ℓ, Cargo
Fleet Chemical Co., Stockton on Tees), PPO 12g; dimethyl POPOP 0.9g
(KochLight).

 These separative procedures were checked by thin layer chromatography
and the percentage recovery was 85% – 90%. Serum lipids were separated
by the same method and samples run on thin layer chromatography before
measurement of radioactivity.

 The rates of fatty acid synthesis are expressed as μmole of fatty
acid synthesized. This was calculated as suggested by Windemeuller and
Spaeth (1966; 1967):

$$\frac{\text{d.p.m. }^3\text{H in fatty acid}}{\text{specific activity }^3\text{H}_2\text{O (d.p.m./}\mu\text{g atom H})\times 13.3}$$

Analysis of fatty acid after ^{3}H$_2$O experiements by sequential degradation
(Foster and Bloom, 1963) and mass spectrophotometric measurements (Wadke, M.
et al, 1973) has shown that an average of 13.3 H atoms per 32 carbons are
labelled with ^{3}H.

Sterol synthesis was calculated with a figure of 19.8 which arose from the
assumption of 45 carbon bound hydrogen atoms (Windemeuller and Spaeth 1966; 1967)

(d) TIME COURSE OF THE APPEARANCE OF SERUM ^{3}H-TRIGLYCERIDE FATTY ACIDS

 Lean and obese 6 week old animals were used. All animals were injected
4 mCi ^{3}H$_2$O into the tail vein and animals bled at 0, 15, 30, 45 and 60
minutes after the injection.   The serum lipids were extracted in chloro-
form:methanol (500 μℓ of the serum in 10 mℓ of chloroform:methanol (2:1)).
This extract was washed with 4 mℓ distilled water. The aqueous phase was
pipetted off and the solvent phase evaporated to dryness. The lipids were
then dissolved in 500 μℓ of chloroform and then spotted on silica gel thin

layer plates, dried and then run in a solvent system of hexane:diethyl ether and formic acid (80:20:2). The spots were developed in iodine vapour and marked. The iodine was removed by placing the plates in a warm place and then the marked spots scraped into scintillation vials and counted in 10 mℓ of scintillant. Vials in which the solvent phase was evaporated were also counted to quantify for any lipids that could not be picked up for spotting.

Duplicate samples from each rat were used for assay at each point.

(e) (i) HALF LIFE OF SERUM TRIGLYCERIDE

This was measured after the injection of Intralipid, a stable soya bean oil emulsion (Vitrum, Stockholm, Sweden) by the method described by Boberg et al (1969). Lean and obese 6 week old animals were anaesthetised with 60 mg/kg nembutal. Tracheotomy was performed on all animals. The jugular vein and the carotid artery were dissected out and then cannulated. After cannulation a blood sample (300 µℓ) was withdrawn via the carotid artery to obtain triglyceride levels before the start of the experiment. Immediately after this the animals received 300 µℓ of Intralipid through the cannula planted in the jugular vein. A blood sample (300 µℓ) was then withdrawn every 5 minutes starting from zero time to half an hour after the Intralipid injection. At every withdrawal care was taken to ensure that no blood already present in the cannula was withdrawn for triglyceride sampling.

The half life was then measured by plotting the log of triglyceride as mg/100% against time and using the slope of the line for the half life.

(ii) SERUM TRIGLYCERIDE EXTRACTIONS

Serum triglycerides were extracted as follows:

(1) To 1.9 mℓ of 99% Isopropanol 100 µℓ of serum was added.

(2) Mixed for 30 seconds.

(3) 2 g of Zeolite mixture (Technicon Ltd) was added to absorb molecules which interfere with the assay (like glucose and glycerol).

(4) After intermittent mixing for 10 min. the zeolite was sedimented by centrifugation at 2000 x g for 5 minutes. The resultant isopropanol solution was assayed for triglyceride using an automated technique

(Technicon Technical Publication AA11-23, 1971) and performed on a
Technicon Autoanalyser II.

(f) THIN LAYER CHROMATOGRAPHY

Glass plates (20 cm x 10 cm) were spread with silica gel G (10g in
15 mℓ distilled water/plate) and then activated by placing in the oven for
at least two hours. After spotting the plates were dried and run in a
solvent system containing hexane:diethyl ether:formic acid (80:20:2)
(Christie, 1973). The lipid spots were made visible by exposure to iodine
vapour and marked.   Plates were then left in a warm place for removal of
iodine, spots scraped off the plate, scintillant added and counted.

		Typical R_f Values
	cholesterol esters	0.95
	Triglycerides	0.66
	Free fatty acids	0.39
	Cholesterol	0.26
	Complex lipids	
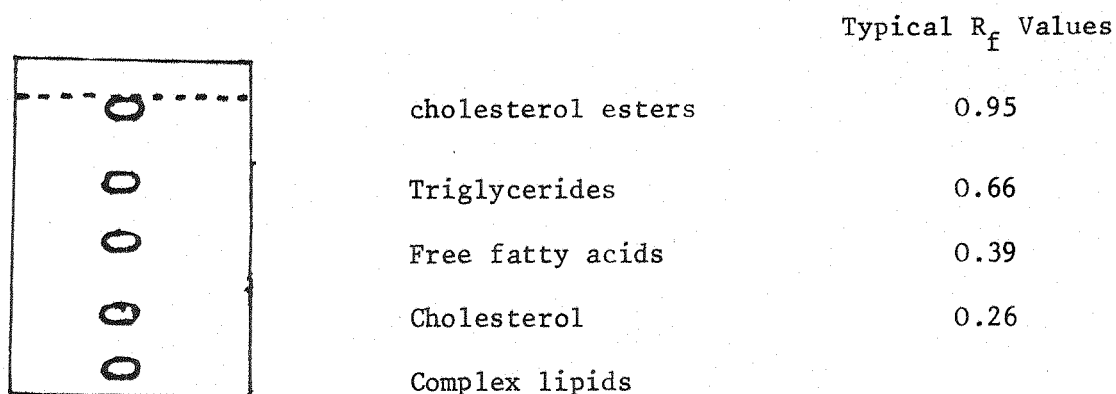
(g) SPECIFIC ACTIVITY OF TISSUE WATER

The calculation of fatty acid synthesis after the intravenous injection
of ^3H_2O assumes that the tissue water pool instantly attains the specific
activity of serum water. To check this assumption the specific activity
of the tissue water of the three sites, i.e., the liver, perimetrial and
subcutaneous fat pads were calculated.

Lean and obese 6 week old animals were used. All animals received
4 mCi of ^3H_2O into the tail vein. Animals were killed at 0 time (immediately
after the injection), 15, 30, 45 and 60 minutes after the injection. A
sample of blood was taken for serum activity, for which 10 µℓ of the serum
was counted in 10 mℓ of scintillant. The liver, perimetrial and subcuta-
neous fat pads were removed and a weighed quantity of each was dried at
$90^{\circ}C$ to ascertain the total tissue water content. A second weighed sample
of each tissue was homogenised in sodium phosphate buffer (pH 7.4). The

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aqueous extract was lyophilized and the water collected in a tube placed in liquid nitrogen.

The ^{3}H content was assayed by scintillation counting. The specific activity of the tissue water was calculated using the total tissue water content and the results expressed as a percentage of the total serum activity.

(h) ENZYME ASSAYS

   (i) Glucose - 6 - phosphate dehydrogenase (G6PDH)
       Julian and Reithel (1975)

$$\text{D glucose} - 6 - \text{P} + \text{NADP}^{+} \xrightarrow{\text{G6PDH}} \text{D gluconate} - 6 - \text{P} + \text{NADPH} + \text{H}^{+}$$
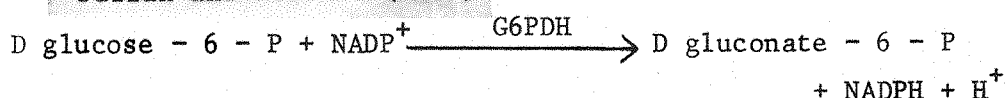
G6PDH (E.C. 1.1.1 49) was assayed on the 10,000g supernatant of a 10% (w/v) homogenate of liver and 20%(w/v) homogenate of adipose tissue in 50 mmole/ℓ Tris, 5 mmole/ℓ MgSO$_{4}$, 1 mmole/ℓ Ethylenediamine tetra acetic acid (K$_{2}$ EDTA) pH 7.6. The tissue homogenate was filtered through glass wool before use. The assay system contained 50 μmoles Tris buffer pH 7.6, 0.2 μmoles NADP, 4 μmoles MgCℓ$_{2}$ in a final volume of 1 mℓ, 10 μℓ of enzyme protein and 0.4 μmole glucose-6-phosphate (Sigma Chemical Co., London) were added and the optical density change at 340 nm followed on a Unicam SP1800 spectrophotometer.

   (ii) Acetyl Coenzyme A carboxylase - Halestrap & Denton (1973)

$$\text{Acetyl- CoA} + \text{CO}_{2} + \text{ATP} \longrightarrow \text{Malonyl} - \text{CoA} + \text{ADP} + \text{Pi}$$

Acetyl CoA carboxylase was assayed on a 45,000 x g supernatant of a 10% (w/v) homogenate in 100 mM phosphate buffer pH 7.5 containing 2 mM EDTA and 5 mM glutathione. The assay included a 30 minute prior incubation of 400 μℓ of the homogenate with 50 μℓ of 200 mM citrate and 200 mM MgCℓ$_{2}$ and 50 μℓ 10% delipidated albumin at 37°C. After the 30 minute incubation, 50 μℓ of this citrate activated aliquot or 50 μℓ aliquot of the crude homogenate were added to 400 μℓ of 0.1 mole Tris Hcℓ pH 7.4 containing 5 mM ATP, 10 mM MgCℓ$_{2}$, 0.5 mM EDTA, 1 mM glutathione, 10 mg/mℓ delipidated albumin, 15 mM KH^{14}CO$_{3}$ (1μCi/μmole) (Radiochemical Centre, Amersham, England) and 0.15 mM acetyl-CoA. After incubation for 3 minutes at 37°C the reactions were terminated by adding 150 μℓ of 10%

(v/v) perchloric acid. The tubes were spun (1,000 x g) and 500 µℓ of
the supernatant placed in a scintillation vial under a stream of air for
3-4 hrs, before counting the radioactivity fixed in malonyl CoA.

A blank was set up everytime containing no acetyl-CoA and results
were corrected for the small amount of ^{14}C found in the absence of added
acetyl-CoA.

Synthesis of acetyl coenzyme A

To 10 mg of coenzyme A (CoA-SH) (Sigma Chemical Co., London) in 1 mℓ
of 0.1 M KHCO$_3$, 10 µℓ of acetic anhydride was added, mixed thoroughly and
allowed to stand for 5 minutes at room temperature. 1 M Hcℓ was added
to adjust the pH between 1 and 2. The aqueous phase was extracted 5 times
with 1 mℓ of ether and then pH adjusted between 5 and 6 with 1 M KHCO$_3$.
Nitrogen was bubbled through the solution until no smell of ether remained.
The acetyl-CoA solution was stored at -10°C.

Delipidation of albumin - Chen (1967)

To 7 gm of Bovine Serum Albumin (Pentex Chemicals Ltd.) 70 mℓ of
distilled water was added. 3.5g charcoal was added and the pH adjusted
to 3.0 with 0.2 M Hcℓ. The mixture was allowed to mix in an ice bath
for 1 hr., then centrifuged at 30,000g for 20 minutes at 2°C. The super-
natant was removed and the pH adjusted to 7.0 with 0.2M NaOH and then
freeze dried over night.

(i) LOWRY PROTEIN ASSAY

A. 2% Na$_2$CO$_3$ in 0.1 M NaOH.

B. 1% CuSO$_4$ and 2% Na K Tartarate.

C. 10% Sodium Deoxycholate.

A:B and C mixed in a ratio 10:0.2:1 just before use.

Assay

(1) 1.1 mℓ of ABC mixture was added to 10 µℓ of liver homogenate and
     20 µℓ of adipose tissue homogenate (1 gm tissue homogenised in 10 mℓ
     100 mM phosphate buffer, 7.5 pH).

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(2)   Rotamixed.

(3)   Allowed to stand at room temperature for 10 minutes.

(4)   100 µℓ of Folin Ciocalteau Reagent (1:1 dilution in distilled water)
      added and rotamixed.

(5)   Allowed to stand at 37°C for 10 minutes.

(6)   Read at 750 nm.

(7)   For blanks equal volumes of homogenizing buffer were used.

(j)   INSULIN IMMUNOASSAY

A modification of the method of Hales and Randle (1963) was used.

The principle of the test is the reaction of a fixed quantity of
Insulin Binding Reagent with an excess of the sample of insulin to be
assayed with a constant amount of ^{125}I-insulin. Cold insulin competes
with ^{125}I-insulin for binding sites so that binding of labelled insulin
to the binding reagent is progressively inhibited by increasing amounts
of unlabelled insulin owing to the degree of dilution of the label. After
the reaction is complete the binding reagent bound insulin is separated
from the free insulin and the distribution of the radioactivity determined.

Reconstitution of Reagents

Insulin binding reagent (Wellcome; Wellcome Research Laboratories,
Beckenham, England).

The freeze dried contents of each bottle were reconstituted with 8 mℓ
of deionised water. The material dissolved immediately on mixing by
gentle inversion.

Making of Standard Insulin Solutions

Insulin Standard (Rat insulin standard - Novo Labs., Denmark) was
dissolved to obtain a concentration of 100 µg/mℓ. This solution was
divided into 20 µℓ aliquots which were frozen separately to avoid repeated
thawing and freezing. This stock solution is stable for 1 month at 4°C
and 1 year at -20°C.

This stock solution was then used for making 6 serial dilutions using Buffer B (0.05M phosphate Buffer 7.4 pH + 0.5% Bovine serum albumin + 0.025% thiomersal + 0.9% NaCℓ).

Procedure

The assay was run in triplicates of each sample.

Setting up of the Assay Tubes

(a) Wash blanks : to which 100 µℓ Buffer B was added.

(b) 'Zeros'   : to which 100 µℓ Buffer B was added.

(c) Standard insulin : standard insulin solutions were set with 100 µℓ of the appropriate insulin standards.

(d) Unknowns   : 100 µℓ of the unknown serum samples used.

Addition of the Insulin Binding Reagent

(a) Wash blanks to which 100 µℓ Buffer A (0.05M phosphate buffer pH 7.4 + 0.5% Bovine serum albumin + 0.025% thiomersal) was added; these serve as controls of the decanting procedure.

(b) 100 µℓ of the reconstituted binding reagent was then added to the zeros, standards and sample tubes. The contents of each tube were mixed on a vortex mixer and then the tubes placed to incubate at $4^{o}C$ for 6 hrs.

Addition of Labelled Insulin

100 µℓ ᴸ the working solution of iodinated insulin ^{125}I (Radiochemical Centre, Amersham, England) was added to each tube and three other tubes as 'totals' containing 100 µℓ each of the iodinated insulin included. All tubes were mixed and left at $4^{o}C$ for 18 hours.

Separation of the Precipitate

All tubes were centrifuged at 2,000G for 20 minutes and the supernatant from all tubes except the totals decanted. Each tube was counted for one minute in the Beckman Instruments γ counter of 80% efficiency for ^{125}I.

Calculation of Results

(a) The background count rate was subtracted from all other counts.

(b) Each count expressed as a percentage of the mean total count.

(c) The mean percentage bound for each set of triplicates determined.

(d) A graph of mean percentage bound versus standard concentration plotted and the curve used to determine the level of insulin in the test samples.

All results are expressed as μu of insulin/mℓ serum sampled.

A typical standard curve for displacement of ^{125}I-insulin by increasing concentrations of standard rat insulin is shown in Fig. 1.

(k) <u>INDUCTION OF DIABETES WITH STREPTOZOTOCIN</u>

Diabetes was induced by an intravenous injection of a freshly prepared solution of streptozotocin (65 mg/kg body weight) in citrate buffer (0.05 mole/ℓ, pH 4.3). Control animals received an equal volume of saline.

Streptozotocin consists of a 2 deoxy-D-glucose with a N-nitromethyl urea side chain at the second carbon atom (2-deoxy-2-(3 methyl-3-nitroso ureido)-D-glucopyranose) (Herr et al, 1967).
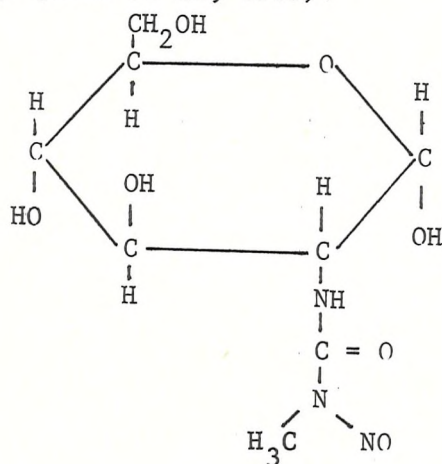
The actual mechanism of streptozotocin action on the β cells of the islet is not known. However it is thought to mediate β cell necrosis from within the β cell by inhibiting the synthesis of pyridine nucleotides. A decrease in the concentrations of oxidised and reduced nicotinamide adenine dinucleotide (NAD and NADH) has been reported after an intravenous streptozotocin injection (Gunnarsson et al, 1974).
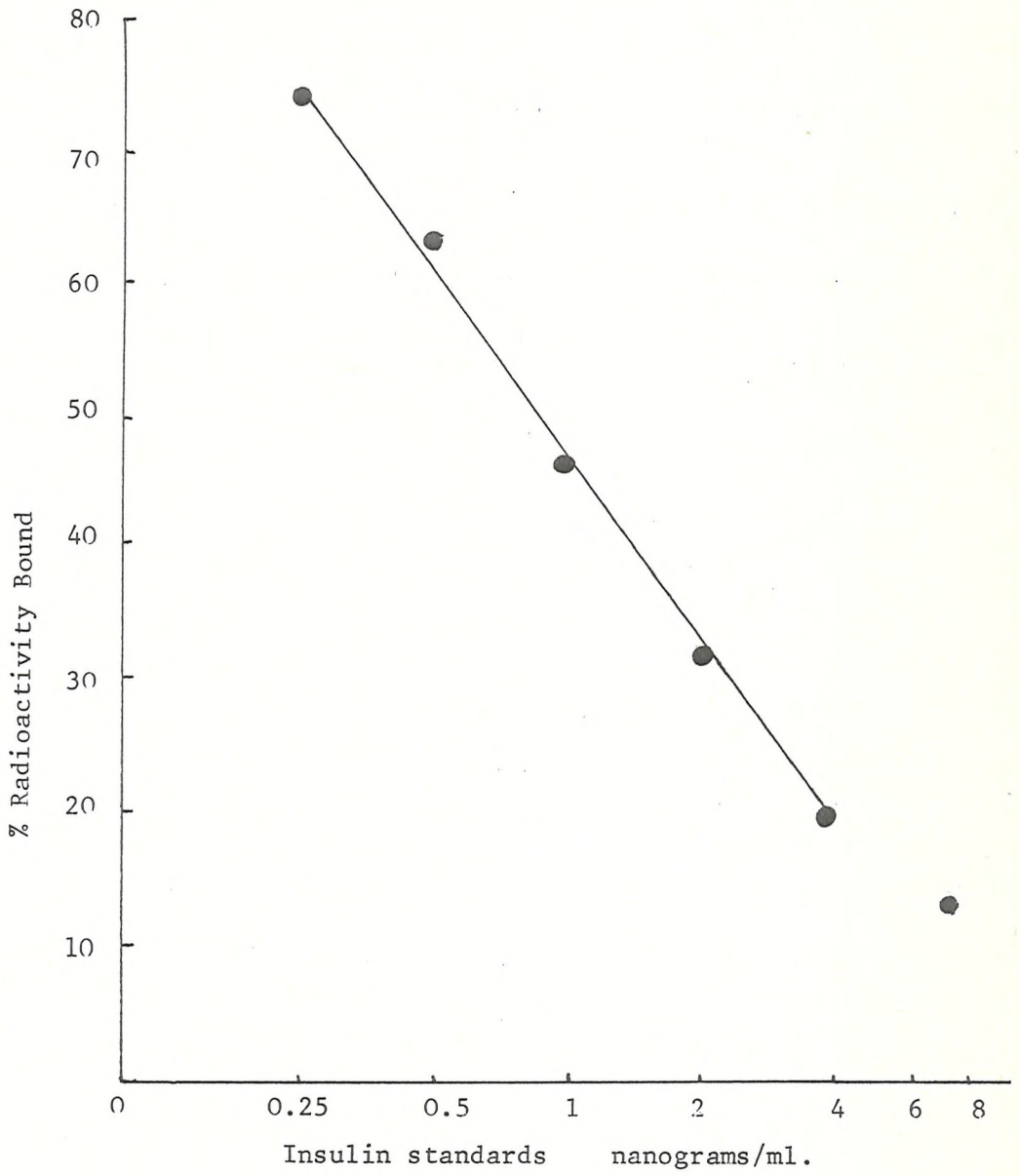
Fig. (i)   Standard curve for insulin.

It is thought that the glucose residue in the streptozotocin molecule may potentiate the β cytotoxic action of this drug by enhancing the uptake of streptozotocin into the islet cells where the cytotoxicity of methylnitrosourea can exert its full effect (Gunnarsson et al, 1977 and Rossini et al, 1977). The D-glucose of streptozotocin could probably exist in a pyranose form with 2 anomers at C-1 similar to those of D-glucose, i.e., α streptozotocin anomer and β streptozotocin anomer. The α anomer has been shown to be more potent than the β anomer at doses between 30 and 45 mg/kg body weight (Rossini et al, 1977).

All streptozotocin treated rats were studied for weight gain over the next 8-10 days during which their urine was sampled for glucose using Clinistix. Then hepatic and adipose tissue lipogenesis were studied.

(1) (i) FUNCTIONAL HEPATECTOMY

In some experiments it was necessary to isolate the liver from the general circulation to prevent hepatic lipogenesis and thus the subsequent transport of newly synthesised lipids to the adipose tissue. Animals were anaesthetised with nembutal (60 mg/kg body weight) and then the hepatic portal vein and the hepatic artery were ligatured. In the controls ligatures were placed around these vessels but not secured (Loten et al, 1974). Soon after these procedures animals were injected with 4 mCi ^3H_2O for measurement of fatty acid synthesis.

(ii) MEASUREMENT OF SERUM GLUCOSE LEVELS

Glucose was measured by an automated method on a Technicon Auto-analyser II (Clinical Method No. 507-72G) adapted from Trinder (1969). It used the reactions:

Glucose + glucose oxidase + oxygen $\longrightarrow$ gluconic acid + H_2O_2

H_2O_2 + peroxidase + chromogen $\longrightarrow$ coloured product.
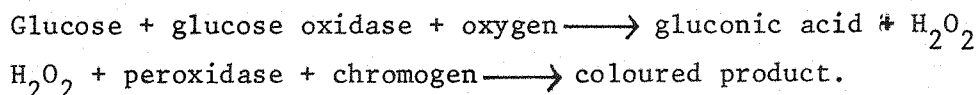
Hence the glucose concentration can be determined colourimetrically.

The samples for the autoanalyser were prepared by diluting the serum 1:9 with distilled water.

(m) ADRENALECTOMY

Animals were bilaterally adrenalectomized under Nembutal (60 mg/kg body weight) anaesthesia using the dorsal approach. The adrenals were easily identified and carefully extirpated in order not to damage the capsule. In the sham operated controls the adrenals were exposed, manipulated and left intact. The wounds were stitched and following adrenalectomy animals were given 0.9% (w/v) sodium chloride. Animals were allowed free access to food for the following two weeks during which body weight gain was followed and at the end of this period hepatic and adipose tissue lipogenesis followed.

At sacrifice all rats were examined for regenerated ectopic adrenal tissue. From such observations some obese rats were designated as unsuccessfully adrenalectomised and these weighed more than the successfully adrenalectomised obese rats.

(n) MEASUREMENT OF RECTAL TEMPERATURE

The measurement of rectal temperatures for identifying pre-obese animals reported by Trayhurn et al (1977) was used. When this method was used for its validity in identifying pre-obese rats before visual differentiation, temperatures on suckling rats were recorded daily and those that showed lower rectal temperatures were marked and growth pattern of all rats followed. At all times the rats that were marked for lower temperatures could later be visually identified as obese at the end of the study period.

All suckling rats were separated from the mother and placed in an open cage for 15 minutes before measurement of temperature. A thermistor (Light Labs., Brighton, England) was inserted a constant distance (11 mm) into the rectum of all animals. After stabilization (5-7 seconds) the temperature was recorded from a Temperature Recorder (15-45°C range) (Light Labs, Brighton, England). All temperatures were measured between 10.00-11.30 hours each day.

Temperatures of animals younger than 15-16 days could not be recorded successfully due to the size of the thermistor.

32.

(o) MEASUREMENT OF ADIPOCYTE CELL DIAMETER

A small piece of the adipose tissue was incubated in a collagenase (Sigma type II) 1 mg/ml in Krebs Ringer Bicarbonate and 2% Bovine Serum Albumin for 30 minutes at 37°C.

A small quantity of the liberated cells were placed on a siliconised glass slide and the diameters of about 100 cells were measured under a calibrated eye piece at a magnification of 10 times.

Calculation

Fat cell volume was calculated according to the formula proposed by Goldrick (1967) as outlined below.

$$\text{Fat cell volume} = \frac{\pi}{6} (3\sigma^2 + \bar{x}^2)\bar{x}$$

where σ^2 = variance of diameter

$\bar{x}$ = mean diameter.

Statistical Analysis of Results

Calculations were performed on a programmable desk top computer (Hewlett-Packard 9810A).

The statistical significance of experimental observations were determined by the unpaired Student's 't' test.

RESULTS

The use of ^3H_2O for assay of lipogenesis is preferable to the use of ^{14}C labelled substrates because of the problems of differing pool sizes between different groups of animals and variations of pool sizes during the course of an experiment. However, this method assumes that the tissue water pool instantly attains the specific activity of serum water. Initial experiments were performed to follow the time course of the rise in the specific activity of tissue water expressed as a percentage of the serum specific activity. Figure 1 shows that this assumption was not correct, because after a very rapid initial exchange (first 15 minutes) tissue water of the liver, the subcutaneous and the perimetrial fat pads only slowly approached the specific activity of serum. The time course for all three tissues in both lean and obese animals was very similar. Therefore since the serum specific activity at 1 hour is used for calculating results the quantitative values will all be slightly underestimated. However, the qualitative interpretation of the results will still be valid.

Figure 2 shows that the rate of hepatic fatty acid synthesis remained roughly linear for one hour and after this there was no further accumulation of ^3H fatty acid indicating that the rate of secretion of newly synthesized fatty acids from the livers now equals the rate of synthesis.

Lipogenesis in the ad. libitum fed fatty rat.

The genetically obese fatty rat (fa/fa) deposits excessive quantities of fat in both subcutaneous and intraperitoneal fat stores. However, little is known of the relative importance of the liver and adipose tissue of fatty rats allowed free access to food. In vitro studies (York & Bray, 1973) have shown that the adipose tissue lipogenesis is increased in the young fatty rats but returns to normal in old fatty rats when insulin resistance has increased.

Hems et al (1975) have shown from in situ studies that the adipose tissue is the major site of excess fatty acid synthesis in the obese ob/ob mouse. To investigate the same in the fatty rat, in vivo experiments were performed on young (5-6 weeks) and older (13 weeks) fatty rats to find out the major site of lipogenesis and the contribution of the liver and adipose
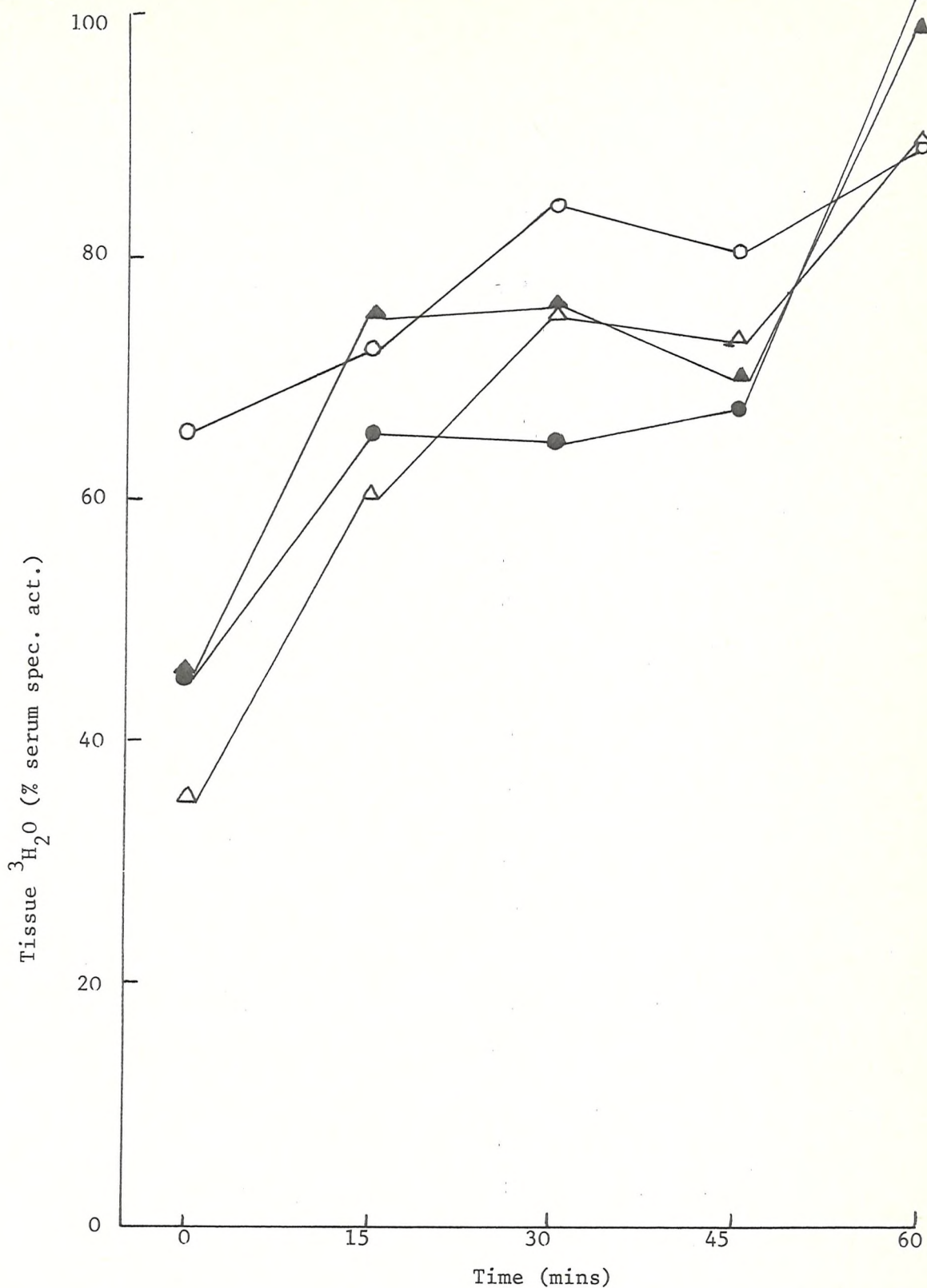
Fig. 1.   Specific activity of tissue water after IV injection of ^3H_2O.

The results are expressed as a % of serum specific activity.
Duplicate samples of each tissue from a single animal were used at
each point. Open symbols lean; solid symbols fa/fa; Liver (o, ●)
and adipose tissue (△, ▲). Perimetrial and subcutaneous fat depots
gave very similar results and the figures for adipose tissue repre-
sent the mean of these two sites at each time.
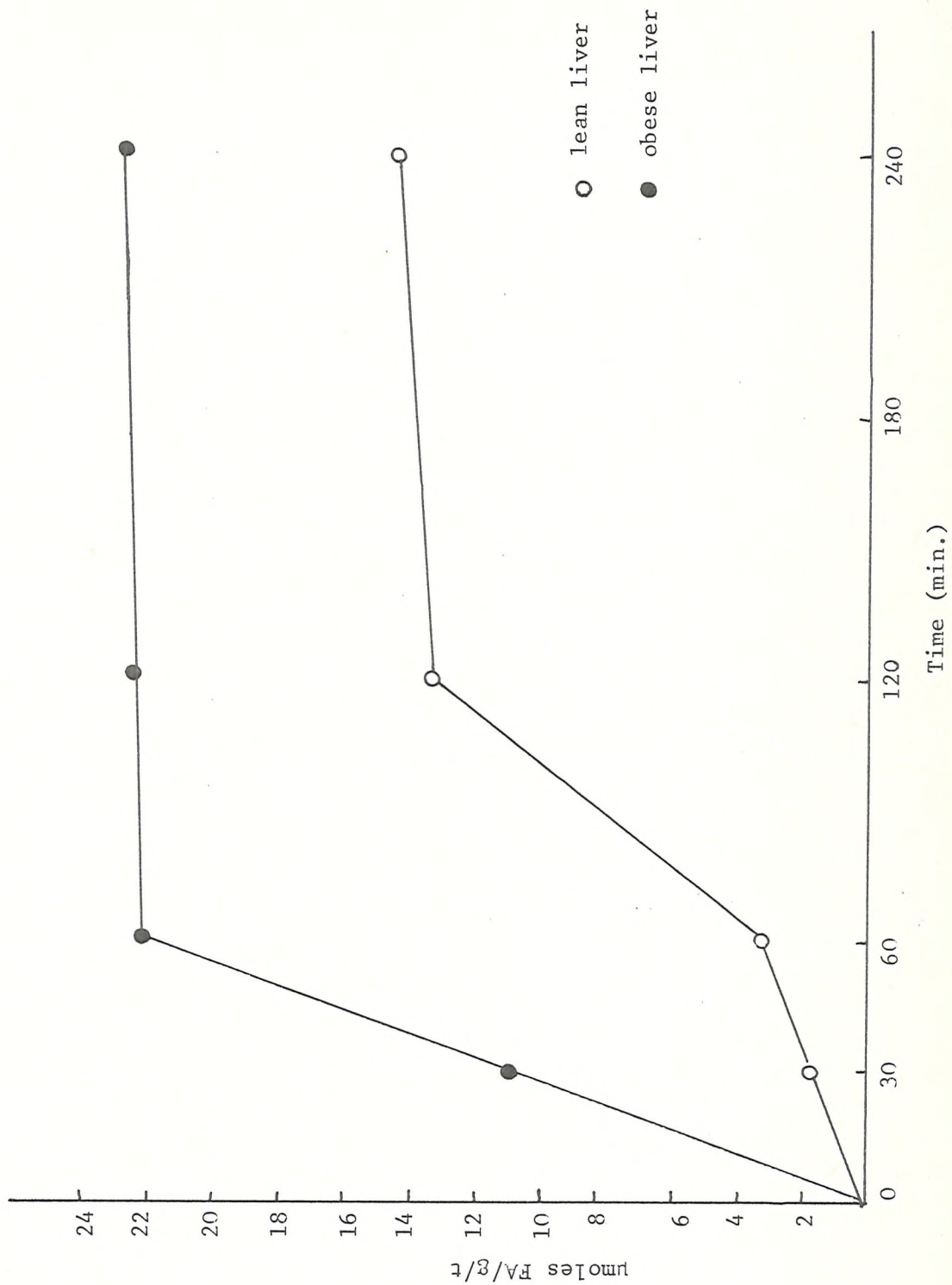
Fig. 2. Time course of hepatic F.A. synthesis in 6 week old rats.

tissue to the total fatty acid synthesis in the fatty rat.

Serum insulin level (Table 1) showed a three fold increase in 5 week old obese rats compared to their lean littermates and it increased further by 13 weeks so that the insulin level in the obese 13 week old rats was five fold higher compared to 13 week old lean rats. The obese 5 week old rats were also hyperphagic compared to their lean littermates. Food intake was not measured on the 13 week old rats in this study but other studies have shown that the obese rats are still hyperphagic at this age (Bray & York, 1972).

The rates of fatty acid synthesis in the liver and the two adipose tissue sites, perimetrial and subcutaneous, in lean and obese rats expressed as μmoles fatty acids synthesized per gram tissue are given in Table 1. At 5 weeks of age the rate of fatty acid synthesis in all three sites of obese rats was approximately ten fold greater than in the lean controls. With increasing age the rates of hepatic fatty acid synthesis increased in lean rats while adipose tissue lipogenesis remained constant. In contrast fatty acid synthesis in the adipose tissue of fatty rats fell dramatically by 13 weeks while hepatic lipogenesis was increased two fold as compared to five week obese rats.

These results of increased hepatic and adipose tissue lipogenesis in the 5 week fatty rat are further supported by the increased glucose-6-phosphate dehydrogenase (G6PDH) and acetyl-CoA-carboxylase (ACC) activities (Table 2) G6PDH activity was increased 3.5 fold in the liver and 2 fold in the adipose tissue of fatty rats compared to their lean littermates. ACC was assayed both before and after activation with 20 mM citrate. In the absence of citrate there was a 3-4 fold greater activity of both hepatic and adipose tissue ACC in fatty rats. After citrate activation ACC activity was increased by 2.5 fold in the liver and 3 fold in the adipose tissue of obese rats compared to their lean littermates. This suggests not only increased activity of ACC in the fatty rats but also increased enzyme protein compared to lean rats.

The radioactivity recovered in the total lipids and sterols is increased in the fatty rats, however the ratio of fatty acids to sterols in 6 week obese rats is 7 for the liver and 15 for the adipose tissue whereas this ratio in lean age matched rats is approximately 2.5 for the liver and adipose tissue. In the 13 week fatty rats this fatty acid to sterol ratio is 17 for the liver and 4 for the adipose tissue compared to 3 for the liver

Table 1 Hepatic and adipose tissue lipogenesis in lean and obese rats fed ad libitum (Mean ± S.E.)

	LEAN		OBESE	
AGE (weeks)	5	13	5	13
TOTAL LIPIDS (μg atoms ^{3}H incorporated into Lipid/gm/h.)			***	***
Hepatic	353 ± 131	380 ± 40	1038 ± 117a	1442 ± 151
P.M.^{x}	46 ± 7	50 ± 8	239 ± 70**b	62 ± 17
S.C.^{xx}	84 ± 9	70 ± 16	184 ± 66a	48 ± 13
STEROLS (μmole/g/h)				
Hepatic	1.4 ± 0.3	1.5 ± 0.1	3.7 ± 0.9*	2.9 ± 0.2***
P.M.	0.5 ± 0.1	0.4 ± 0.2	0.7 ± 0.1	0.5 ± 0.2
S.C.	0.2 ± 0.02	0.4 ± 0.1	0.6 ± 0.3	0.3 ± 0.1
FATTY ACIDS (μmole/g/h)			***	
Hepatic	2.7 ± 0.5b	4.8 ± 0.5	26.5 ± 3.5a	47.0 ± 6.6***
P.M.	1.4 ± 0.1	1.2 ± 0.4	10.6 ± 2.2***d	2.1 ± 0.2
S.C.	0.7 ± 0.2	1.1 ± 0.3	7.6 ± 1.4***c	1.0 ± 0.1
SERUM INSULIN (μu/ml)				
	56 ± 5	67 ± 5	164 ± 22**	319 ± 22***
P		N.S.		<0.01
FOOD INTAKE (g/d)				
	15.8 ± 2.5		20.9 ± 1.9	
P		<0.1		

12 animals in each group.

x - perimetrial fat;   xx - subcutaneous fat

[a]$p < 0.05$;   [b]$p < 0.02$;   [c]$p < 0.01$;   [d]$p < 0.001$ compared to older animals of same group.

*$p < 0.02$;   **$p < 0.01$;   ***$p < 0.001$ compared to age matched lean animals.

Table 2   Hepatic and adipose tissue enzyme activities in lean and obese rats fed ad libitum (Mean + S.E.)

AGE (weeks)	G6PDH (mμmoles/mg protein/min)		Acetyl CoA carboxylase (mμmoles/mg protein/min)			
			Hepatic		S.C.^{x}	
	Hepatic	S.C.^{x}	+ citrate	-	+ citrate	-
LEAN 5	50.0 + 7.5	30.0 + 1.8	8.0 + 0.3	0.8 + 0.2	10.0 + 0.6	3.0 + 0.6
OBESE 5	180.0 + 20.0	60.0 + 15.0	19.0 + 5.0	3.0 + 0.5	35.0 + 2.8	8.0 + 0.8
P	<0.001	<0.05	<0.05	<0.001	<0.001	<0.001

8 animals in each group

x - subcutaneous fat

G6PDH - glucose-6-phosphate dehydrogenase

and adipose tissue of 13 week lean rats. The rate of synthesis of fatty acids in the liver of old and young obese rats and the adipose tissue of 6 week rats is much higher than the rate of sterol synthesis.

The problem of expressing values of fatty acid synthesis as μmoles/ gm.tissue/h. is that in the fatty rats the fat cell size is increased. The major part of this increase is accounted for by the increase in size of the intracellular triglyceride stores. Hence a fall in fatty acid synthesis expressed per gram tissue could merely reflect this increase in weight of triglyceride stored. In addition the subcutaneous and perimetrial fat pads of fatty rats are characterised by an increase in fat cell number. So to correct for differences in the size and number of cells the total rate of synthesis for the whole tissue has been calculated. These results show the true increase in total lipogenesis in fatty rats (Table 3). So that the total hepatic lipogenesis in 5 week fatty rats showed a seventeen fold increase with the adipose tissues resulting in a 60-70 fold increase compared to 5 week lean rats. The total hepatic lipogenesis of 13 week fatty rats showed a 15 fold increase whereas the total adipose tissue lipogenesis of the two sites showed only an 8 fold increase in spite of the considerably heavier tissues compared to lean 13 week old rats. Even so the total fatty acid synthesis in both adipose tissue depots fell with increasing age in the fatty rats, showing that the decrease in fatty acid synthesis per gram tissue was not an artifact due to the increased size of adipocytes. The ratio of the total fatty acid synthesised in the liver and adipose tissue: (L/AT) was lower in fatty rats at 5 weeks (2 compared to 7.4 in lean) but had increased to a value above that of lean rats by 13 weeks (10.8 in obese compared to 5.9 of lean).

The ratio of the total fatty acids to the total sterol synthesis in the obese animals shows that though the rate of sterol synthesis increases in the livers and adipose tissues of obese rats compared to lean rats, the increase in fatty acid synthesis in these obese rats is remarkably higher than sterol synthesis.

The ^{3}H fatty acids extracted from the adipose tissue could represent either fatty acids synthesized de novo within the adipose tissue or fatty acids synthesized in the liver and subsequently transported to the adipose tissue in the form of very low density lipoproteins during the time of the experiment. To obtain some indication of the size of this hepatic contribution to the adipose tissue ^{3}H fatty acid experiments were

Table 3 Total hepatic and adipose tissue lipogenesis in lean and
obese rats fed ad libitum (Mean $\pm$ S.E.)

	LEAN		OBESE	
AGE (weeks)	5	13	5	13
TOTAL LIPIDS (μg atoms ^{3}H incorporated into lipids/tissue/h.)				
Hepatic	1808 ± 262	2266 ± 131	7297 ± 655^b	10336 ± 983
P.M.^{x}	25 ± 10	79 ± 33	1127 ± 64^e	498 ± 56
S.C.^{xx}	90 ± 10	100 ± 35	1402 ± 70^e	852 ± 131
STEROLS (μmole/whole tissue/h)				
Hepatic	7.3 ± 2.9	11.8 ± 2.3	30.3 ± 2.0^c	38.0 ± 5.5
P.M.	0.3 ± 0.1^a	0.5 ± 0.2	2.3 ± 0.2^d	3.3 ± 1.0
S.C.	0.3 ± 0.1^a	0.6 ± 0.2	3.4 ± 0.1^d	7.0 ± 2.2
FATTY ACIDS (μmole/whole tissue/h)				
Hepatic	12.7 ± 4.4	39.8 ± 12.0	221.0 ± 58.0	618.0 ± 100.0
P.M.	0.8 ± 0.1	3.2 ± 0.7	49.0 ± 5.4	24.0 ± 1.8
S.C.	0.9 ± 0.2	3.5 ± 0.8	64.0 ± 2.5	32.7 ± 5.5
L/AT	7.4	5.9	2.0	10.8

12 animals in each group

x - perimetrial fat;   xx - subcutaneous fat

L/AT - ratio of total lipogenesis in liver and adipose tissue
calculated from $\dfrac{\text{Hepatic f.a. synthesis}}{\text{P.M. + S.C. f.a. synthesis}}$

$^a p < 0.05$; $^b p < 0.02$; $^c p < 0.01$; $^d p < 0.001$ - compared to older animals of same group.

All values for obese animals are significantly different from lean animals of the same age at the level of $p < 0.001$.

Table 4   The contribution of hepatic fatty acids to the apparent
adipose tissue lipogenesis in lean and obese rats fed ad
libitum (Mean + S.E.).

	LEAN		OBESE	
	Control	Hepatectomised	Control	Hepatectomise
FATTY ACIDS (μmole/whole tissue/h)				
Hepatic	17.2 + 3.9	N.D.	199.2 + 20.0	N.D.
P.M.[x]	1.7 + 0.3	1.5 + 0.5	41.4 + 11.7	4.0 + 1.4*[a]
S.C.[xx]	1.5 + 0.2	1.5 + 0.4	48.0 + 9.6	6.8 + 2.0*[a]
SERUM INSULIN (μu/ml)				
	64 + 5	31 + 4	141 + 24	57 + 5
p		<0.02		<0.05
SERUM GLUCOSE (mmol/ℓ)				
	9.4 + 0.4	2.3 + 0.4	9.5 + 0.3	2.8 + 0.4
p		<0.001		<0.001

6 animals in each group

x - perimetrial fat;   xx - subcutaneous fat

N.D. - [3]H d.p.m. in fatty acids could not be distinguished from
background d.p.m.

* p < 0.05 compared to control animals of same group

[a] p < 0.05 compared to appropriate lean group
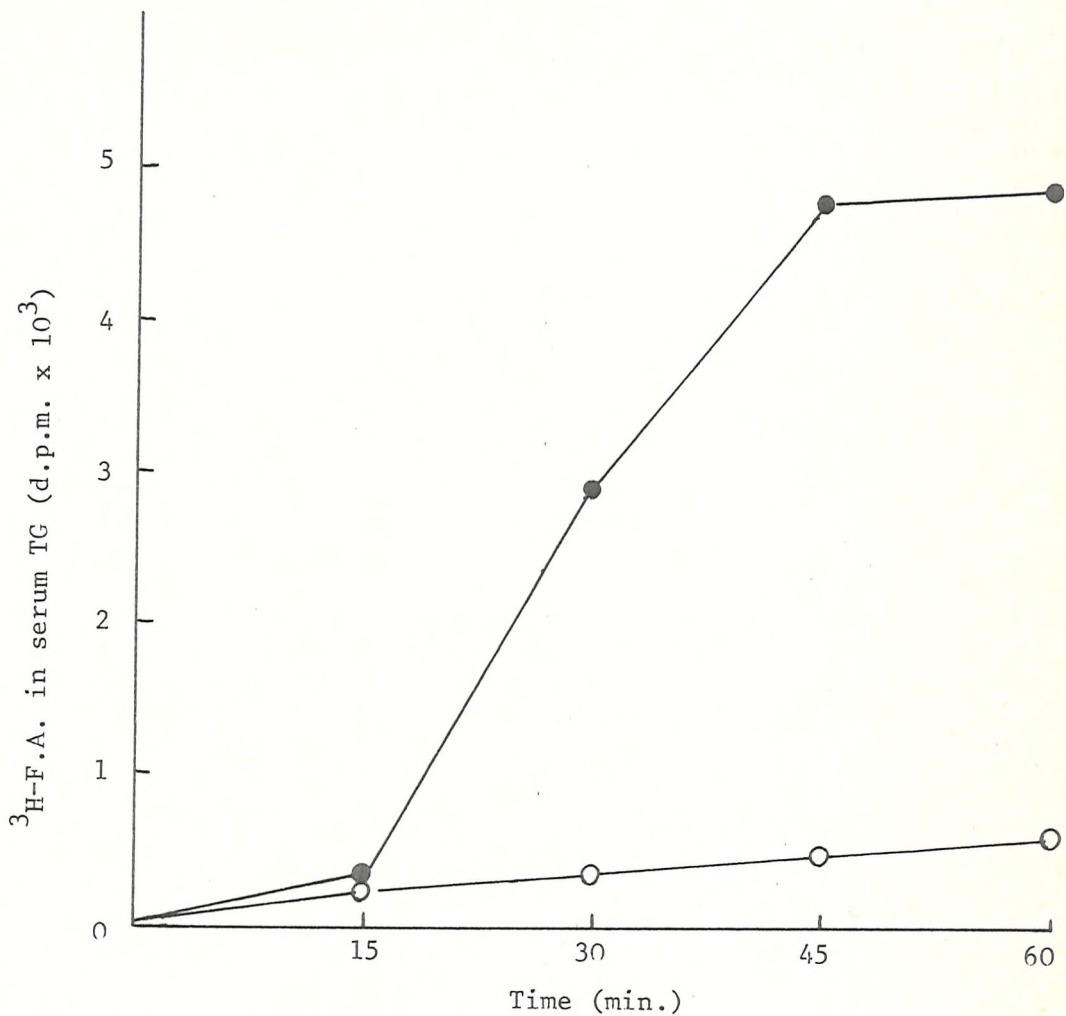
Fig. 3. Time course of the appearance of serum ^{3}H triglyceride-fatty acids following the injection of ^{3}H$_2$O into (lean o) and fa/fa (●) rats. Duplicate samples from a single rat were used for assay at each time point.
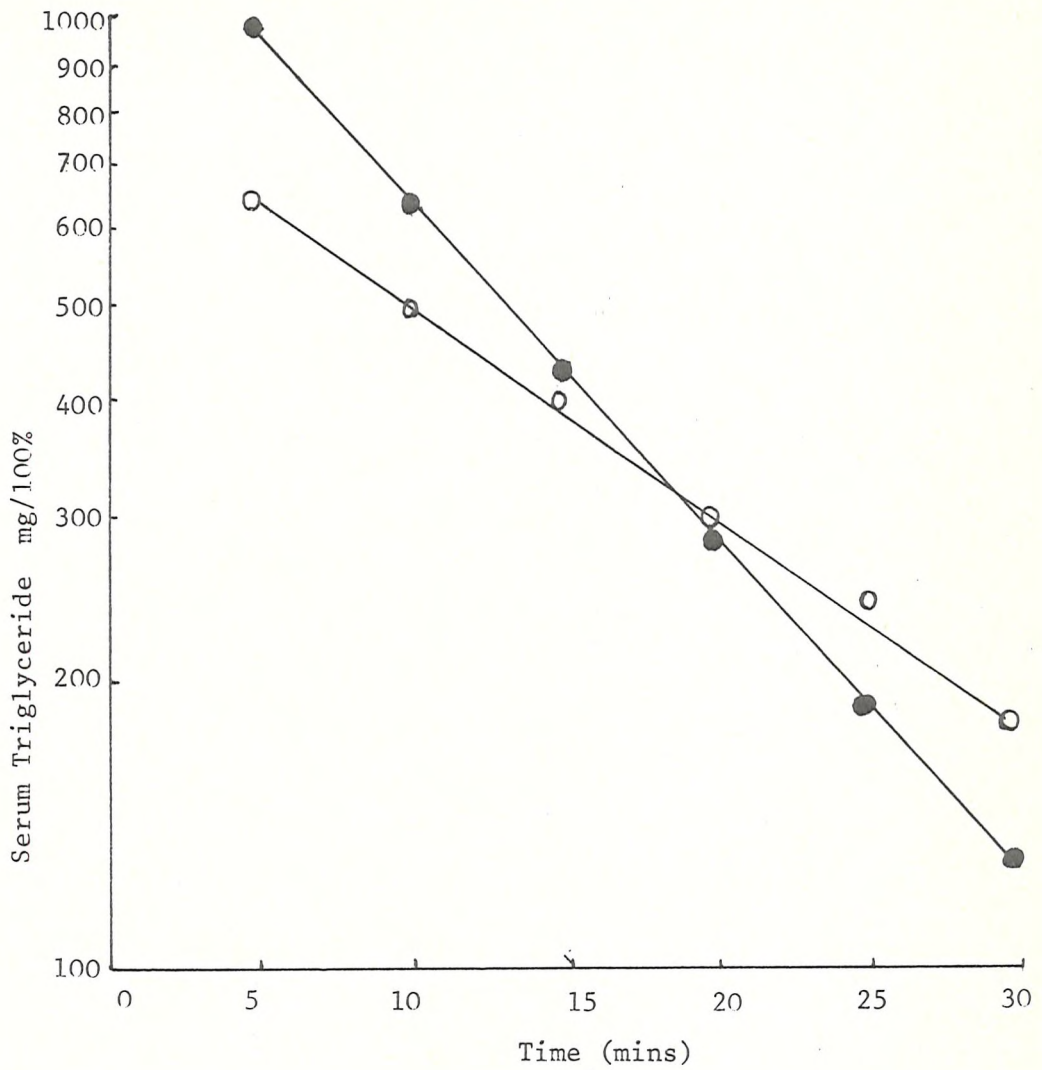
Fig. (4)   Disappearance of injected Intralipid.
300 μℓ of intralipid was injected into the rat and
blood sampled every 5 mins after the injection for
the disappearance of the injected intralipid.

● obese;   ○ lean.
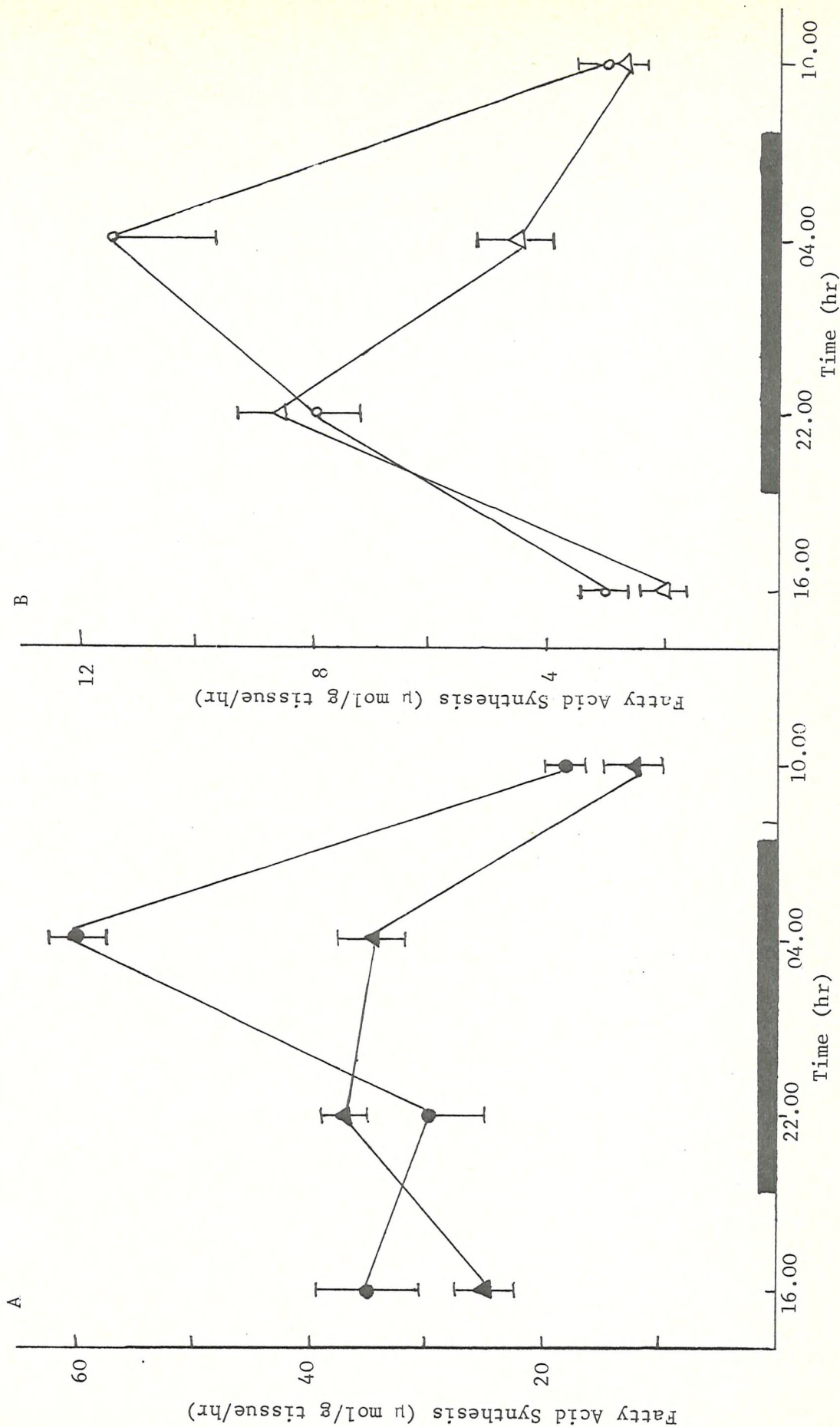
Fig. 5. Diurnal variation of lipogenesis in 6 week old lean and obese fa/fa rats. Lipogenesis in fa/fa (A) and lean (B) rats allowed free access to food was measured by the incorporation of ^3H_2O into hepatic (O,●) and subcutaneous fat (△, ▲). Values for perimetrial adipose tissue did not differ from subcutaneous fat and so have been omitted. Three animals were used at each time.

repeated using 6 week old functionally hepatectomized rats. In such lean and obese animals (Table 4) there was no significant incorporation of ^{3}H into hepatic lipids. The accumulation of ^{3}H fatty acids was similar in adipose tissue of lean controls and hepatectomized rats. However, in the fatty hepatectomized rats 80%-90% of the ^{3}H fatty acid in the adipose tissue was lost, suggesting that hepatic secretion of ^{3}H fatty acids was a major source of the ^{3}H label in adipose tissue of the fatty rats. Serum insulin and serum glucose in hepatectomized rats were reduced to a similar degree in lean and fatty rats.

Although no significant activity of ^{3}H-triglyceride-fatty acids were observed in the serum of lean rats during the experimental time course (figure 3) the serum triglyceride of fatty rats was rapidly labelled. After a lag period of 15 to 30 minutes the activity of ^{3}H triglyceride fatty acids reached a plateau by 45-60 minutes. Also the half life of serum triglyceride was measured by the fall of serum triglyceride accumulation after the injection of Intralipid (figure 4) was only 7.6 $\pm$ 0.4 minutes in fatty rats as compared to 12.5 $\pm$ 0.5 minutes in age matched 6 week lean littermates.

All the previous experiments were performed between 10.00 and 11.00 hours each day. Since the rat is a nocturnal animal, it was interesting to study the diurnal variation in fatty acid synthesis (figure 5). As expected both hepatic and adipose tissue fatty acid synthesis were greater in obese and lean rats during the dark period (between 7 p.m. and 7 a.m.) although there was some divergence as to the actual time of peak lipogenesis. As measurements were only made at 6 hour intervals it is possible that peak lipogenesis may have occurred in both tissues at some time between the measurements at 22.00 and 04.00 hours.

Effect of feeding a high sucrose diet

Sucrose feeding results in an increased rate of fatty acid synthesis because the rate of glycolysis of fructose is greater than that for glucose therefore providing more substrate for fatty acid synthesis and so forming a better precursor of fatty acids.

Animals were weaned onto a high sucrose diet and allowed free access to the diet for two weeks after which they were sacrificed. Sucrose feeding

(Table 5) resulted in a two fold increase in hepatic lipogenesis and a three fold increase in adipose tissue lipogenesis of lean rats compared to lean rats fed the rat chow. This increase was even greater when lipogenesis was expressed for whole tissue because sucrose feeding caused an increase in tissue weights compared to age matched lean rats fed the ordinary rat chow. However, sucrose feeding of 6 week obese rats resulted in a 1.5 fold increase in hepatic lipogenesis with no change in adipose tissue lipogenesis when compared to obese rats on a rat chow diet. However, insulin levels in both animals had increased on being fed a high sucrose diet, with the obese sucrose fed rats having a three fold higher level compared to lean sucrose fed rats in spite of not being significantly hyperphagic. However, lipogenesis in all three sites remained a lot higher than in the lean rats on the same high sucrose diet. The hepatic G6PDH activity (Table 6) showed approximately a three fold increase in both lean and obese rats when fed the high sucrose diet, whereas hepatic fructoaldolase and fructokinase showed no change in either lean or obese sucrose or chow fed rats. This increased G6PDH activity along with the increased insulin levels in lean and obese rats fed a high sucrose diet support the observed increased hepatic lipogenesis.

Role of hyperphagia

Since hyperphagia in the fatty rat could lead to increased lipogenesis, experiments were performed on obese rats that were pair fed to the food intake of lean littermates. Fatty rats were pair fed to lean rats and meal trained (4 hours daily) to prevent differences in feeding pattern that could arise due to a loss of diurnal rhythm in fatty rats as well as prevent a comparison of nibbling versus gorging. This regime leads to much more reproducible rates of fatty acid synthesis than are obtained when animals receive food ad libitum (Lowenstein, 1971). This period lasted for two weeks starting straight after weaning. On the day of sacrifice animals were injected with ^3H_2O 2 hours after the start of their four hour feeding session and sacrificed an hour later (3 hours after beginning feeding). Under these conditions both the serum insulin and the synthesis of fatty acids in all three sites were greatly enhanced in the lean rats (Table 7). The serum insulin level of the obese rats was not significantly different from the lean rats three hours after the start of the feeding

Table 5   Hepatic and adipose tissue lipogenesis in 6 week old lean and
obese rats fed a high sucrose diet ad libitum (Mean ± S.E.)

| | LEAN | | OBESE | |
	Sucrose	Chow	Sucrose	Chow
FATTY ACIDS (μmole/g/h)				
Hepatic	4.9 ± 0.8^b	2.7 ± 0.5	$38.2 \pm 3.9*^c$	26.5 ± 1.5
P.M.[x]	3.3 ± 1.3	1.4 ± 0.1	$12.9 \pm 0.9*$	10.6 ± 1.1
S.C.[xx]	3.3 ± 0.9^c	0.7 ± 0.1	$10.1 \pm 2.0*$	7.6 ± 0.4
FATTY ACIDS (μmole/whole tissue/h)				
Hepatic	34.4 ± 8.0^a	12.7 ± 4.4	$289.7 \pm 24.4*$	221.0 ± 58.0
P.M.	3.2 ± 2.2	0.8 ± 0.1	$36.8 \pm 3.5*$	49.0 ± 5.4
S.C.	6.9 ± 2.5^b	0.9 ± 0.2	$73.9 \pm 7.2*$	64.1 ± 3.5
SERUM INSULIN (μu/ml)				
	87 ± 13	56 ± 5	$286 \pm 27*$	164 ± 22
P		<0.05		<0.01
FOOD INTAKE (g/d)				
	14.9 ± 1.5	15.8 ± 2.5	16.9 ± 0.7	20.9 ± 1.9
		N.S.		0.1

10 animals in each group were weaned onto a high sucrose diet.

x - perimetrial fat;   xx subcutaneous fat

[a] $p < 0.05$;   [b] $p < 0.02$;   [c] $p < 0.01$ - compared to chow fed
animals of the same group

*$p < 0.001$ compared to appropriate lean group

Table 6 Hepatic glucose-6-phosphate dehydrogenase (G6PDH), fructoaldolase
and fructokinase in 6 week old lean and obese rats fed a high
sucrose or chow diet ad libitum (Mean $\pm$ S.E.)

	LEAN		OBESE	
	Sucrose	Chow	Sucrose	Chow
Hepatic G6PDH (mµmóle/mg protein/min)				
	182.0 ± 10.0	50.0 ± 7.5	420.0 ± 24.0[b]	180.0 ± 20.0
p		<0.001		<0.001
Hepatic Fructoaldolase (mµmole/mg protein/min)				
	13.6 ± 4.1	19.0 ± 5.6	14.2 ± 4.8	23.0 ± 6.7
p		<N.S.		<N.S.
Hepatic Fructokinase (mµmole/mg protein/min)				
	28.0 ± 7.7	28.0 ± 5.5	34.0 ± 4.7	35.6 ± 6.9
p		N.S.		N.S.

10 animals in each group weaned onto a high sucrose diet

[b] $p < 0.001$ compared to appropriate lean group

Table 7 The effect of pair feeding on fatty acid synthesis in obese rats fed a chow diet (Mean ± S.E.)

	LEAN		OBESE	
	Pair fed	Ad libitum	Pair fed	Ad libitum
FATTY ACIDS (μmole/g/h)				
Hepatic	9.2 ± 1.4***	2.7 ± 0.5	12.7 ± 1.0***[a]	26.5 ± 1.5
P.M.[x]	12.4 ± 2.9***	1.4 ± 0.1	10.2 ± 3.9	10.6 ± 1.1
S.C.[xx]	7.0 ± 1.1***	0.7 ± 0.1	3.5 ± 1.4**[a]	7.6 ± 0.4
FATTY ACIDS (μmole/whole tissue/h)				
Hepatic	33.0 ± 7.0*	12.7 ± 4.4	54.2 ± 3.0**[b]	221.0 ± 58.0
P.M.	7.9 ± 2.0***	0.8 ± 0.1	25.7 ± 5.4**[c]	49.0 ± 5.4
S.C.	7.8 ± 1.0***	0.9 ± 0.2	23.6 ± 6.8***[c]	64.0 ± 2.5
SERUM INSULIN (μu/ml)				
	154.0 ± 66.0	56.0 ± 5.0	208.0 ± 17.0	164.0 ± 22.0
p		<N.S.		<N.S.
HEPATIC G6PDH (mμmoles/mg protein/min)				
	33.2 ± 8.0	50.0 ± 7.5	56.9 ± 7.7[b]	180.0 ± 20.0
p		<N.S.		<0.001
FOOD INTAKE (g/d)				
	8.3 ± 0.8	15.8 ± 2.5	8.3 ± 0.8	20.9 ± 1.9
		<0.01		<0.001

12 animals per group were pair fed for 2 weeks from 4 weeks of age on a meal eating 4h regime as described under Methods. ^3H_2O was injected I.V. 2 h after start of feeding period and animals sacrificed 1h later. Fatty acid synthesis for ad lib. fed same as in Tables 1 and 3.

x – perimetrial fat;   x x subcutaneous fat.

*p < 0.02;   **p < 0.01;   ***p < 0.001 compared to ad libitum animals of same group.

[b]p < 0.02;   [c]p < 0.01   compared to appropriate lean group.

period. Furthermore the rates of fatty acid synthesis (μmoles/g. tissue/ h) in pair fed obese rats were reduced to or slightly below those rates seen in the lean rats. Even so the total synthesis of fatty acids (μmoles/ tissue/h) still remained approximately 1.5 fold higher in the liver and three fold higher in the adipose tissue sites in the obese rats because of the already increased tissue sizes. Similarly, pair feeding reduced the hepatic G6PDH activity in the obese rats to a third of the values in ad libitum fed obese rats.

On pair feeding on a meal eating regime a high sucrose diet (Table 8) lipogenesis in all the three sites (liver six fold and adipose tissue ten fold) of lean and obese (liver two fold and adipose tissue three fold) rats increased considerably compared to lean and obese rats allowed to feed ad libitum a high sucrose diet. Similarly serum insulin levels in lean meal fed animals were 3.5 fold increased and in the obese 1.5 fold increased compared to the corresponding ad libitum fed groups. However, on pair feeding there was no significant difference between serum insulin levels, hepatic G6PDH activity or adipose tissue lipogenesis between lean and obese rats pair fed a high sucrose diet. Even so, the rate of hepatic lipogenesis in the obese pair fed rats remained two fold higher compared to the similarly treated lean group, with total hepatic lipogenesis remaining three fold higher with adipose tissue lipogenesis showing a 1.5 to three fold increase in the subcutaneous and perimetrial fat pads of obese pair fed rats.

Role of hyperinsulinemia

Hyperinsulinemia is also associated with increased lipogenesis. To find the major site of insulin action on fatty acid synthesis and the role of insulin in the obese syndrome, experiments were performed on animals in which diabetes was induced by injecting streptozotocin. After streptozotocin injection the weight gain was noted for the following eight days and the presence of glucose in the urine checked by using Clinistix to substantiate the induction of diabetes.

Streptozotocin treatment of weanling (4 week old rats sacrificed at 5 weeks) obese rats reduced the serum insulin level to a fifth of the levels of obese controls so that insulin levels were even lower than those

Table 8: The effect of pair feeding a high sucrose diet on fatty acid
synthesis in obese rats (Mean $\pm$ S.E.)

	LEAN		OBESE	
	Pair fed	Ad Libitum	Pair fed	Ad libitum
FATTY ACIDS (μmole/g/h)				
Hepatic	31.1 ± 2.7*	4.9 ± 0.8	66.8 ± 3.8*[b]	38.2 ± 3.9
P.M.[x]	33.3 ± 1.9*	3.3 ± 1.3	36.1 ± 2.3*	12.8 ± 0.9
S.C.[xx]	31.5 ± 0.9*	3.3 ± 0.9	29.8 ± 1.5*	10.1 ± 2.0
FATTY ACIDS (μmole/whole tissue/h)				
Hepatic	186.6 ± 20.2*	34.4 ± 8.0	536.5 ± 25.5*[b]	289.8 ± 29.1
P.M.	21.9 ± 0.4*	3.2 ± 2.2	66.8 ± 3.4*[b]	36.6 ± 3.5
S.C.	49.9 ± 1.5*	7.9 ± 2.5	80.0 ± 1.8[b]	73.8 ± 7.2
SERUM INSULIN (μu/mℓ)				
	350 ± 29	87 ± 13	357 ± 37	286 ± 27
p		<0.001		N.S.
HEPATIC G6PDH (mμmole/mg protein/min)				
	219 ± 15.6	182 ± 10.0	264 ± 10.5[a]	420 ± 24
p		0.05		<0.001
FOOD INTAKE (g/d)				
	9.5 ± 2.2	15.0 ± 0.5	9.5 ± 2.2	17.0 ± 0.7
p		<0.02		<0.001

12 animals per group were pair fed for 2 weeks a high sucrose diet from 4
weeks of age on a meal eating 4h regime as described under Methods. ${}^{3}H_{2}O$
was injected 1V 2h after the start of the feeding period and the animals
sacrificed 1h later.   Fatty acid synthesis for ad lib fed sucrose diet as in
Table 5.

x - perimetrial fat;   x x - subcutaneous fat.

*p < 0.001 compared to same ad libitum fed group

[a]p < 0.02;   [b]p < 0.001 compared to appropriate lean group.

of lean controls (Table 9). In older obese animals streptozotocin treatment lowered serum insulin levels to approximately a sixth of the obese controls and these values were similar to insulin levels of lean controls. Diabetes resulted in a smaller effect on insulin levels in lean rats (lean diabetic rats showed approximately half the values of lean controls) compared to obese rats which showed a six fold decrease. Hepatic lipogenesis in 5 week old diabetic fatty rats expressed for gram liver or whole liver (Table 10) was less than that of lean controls. The rate of adipose tissue lipogenesis was similar to lean controls but total adipose tissue lipogenesis still remained higher. In the older animals treated with streptozotocin lipogenesis in all three sites was reduced to lean values when expressed either for gram tissue (Table 9) or for whole tissue (Table 10). Similarly weight gain in fatty rats treated with streptozotocin was reduced compared to their equivalent fatty controls.

The lean rats appear to be less responsive to streptozotocin treatment as seen by the smaller reduction in insulin levels as well as a smaller reduction in lipogenesis compared to lean controls. The adipose tissue of both lean and obese rats appears to be less sensitive to the fall in insulin compared to the liver.

Effect of adrenalectomy on the fatty rat

Administration of glucocorticoids (Volpe and Marasa, 1975) in adult rats has been shown to reduce activities of fatty acid synthetase and acetyl-CoA carboxylase in the adipose tissue along with a decrease in fatty acid synthesis with no effect in the liver whereas adrenalectomy produced an increase in activities of these lipogenic enzymes in the adipose tissue with no change in the liver.

To study the significance of adrenocortical steroids in the obese rat, experiments were performed using adrenalectomised rats. Adrenalectomy was performed on lean and obese 5 week old animals and these were sacrificed at 8 weeks.

During the three weeks the rate of weight gain of successfully adrenalectomised obese rats paralleled the rate of weight gain of sham operated lean controls (figure 6) so that at the end of the experiment adrenalectomised

40.

Table 9   The effect of streptozotocin treatment on fatty acid synthesis in obese and lean rats (Mean ± S.E.)

	AGE (weeks)	FATTY ACID SYNTHESIS (μmol/g/h)			SERUM INSULIN (μu/mℓ)	WEIGHT GAIN (g/d)
		Hepatic	P.M.[x]	S.C.[xx]		
Lean Control	5	2.7 ± 1.0	1.4 ± 0.1	0.7 ± 0.1	56 ± 5	1.5 ± 0.3
Lean Strept'n	5	1.9 ± 0.02	1.2 ± 0.1	1.1 ± 0.07	31 ± 8	0.7 ± 0.2
Obese Control	5	26.5 ± 1.0[c]	10.6 ± 1.1[c]	7.6 ± 0.4[c]	164 ± 22	4.7 ± 0.3
Obese Strept'n	5	0.8 ± 0.1[e]	3.8 ± 0.9[d]	1.2 ± 0.4[e]	28 ± 4[e]	1.1 ± 0.2
Lean Control	13	4.8 ± 1.5	1.2 ± 0.1	1.1 ± 0.3	67 ± 5	1.1 ± 0.2
Lean Strept'n	13	2.5 ± 0.01	0.7 ± 0.04	0.9 ± 0.04	44 ± 5	0.8 ± 0.1
Obese Control	13	46.9 ± 10.6[c]	2.1 ± 0.2[b]	1.0 ± 0.1	319 ± 22[b]	4.6 ± 0.8
Obese Strept'n	13	2.4 ± 0.5[e]	0.3 ± 0.1[c,e]	0.1 ± 0.002	55 ± 2[e]	1.5 ± 0.4

8 animals in each group. Lipogenesis was measured 8 days after streptozotocin treatment. Lean and obese control data is identical to that in Table 1. Weight gain is the change between streptozotocin treatment and sacrifice at day 8.

x – perimetrial fat;   xx – subcutaneous fat.

[b] $p < 0.01$;   [c] $p < 0.001$ compared to lean control group;   [d] $p < 0.01$;   [e] $p < 0.001$ compared to obese control.

Table 10   The effect of streptozotocin treatment on total fatty acid synthesis of liver and adipose tissue in obese and lean rats (Mean $\pm$ S.E.)

	AGE (weeks)	Hepatic	FATTY ACID SYNTHESIS (μmol/whole tissue/h) P.M.^{x}	S.C.^{xx}
Lean control	5	12.7 ± 4.4	0.8 ± 0.1	0.9 ± 0.2
Lean strept'n	5	8.1 ± 2.3	0.6 ± 0.1	1.1 ± 0.3
p		N.S.	N.S.	N.S.
Obese control	5	221.0 ± 58.0	49.0 ± 5.4	64.1 ± 2.5
Obese strept'n	5	6.7 ± 1.5	12.4 ± 3.5^a	4.8 ± 3.2
p		<0.01	<0.001	<0.001
Lean control	13	39.8 ± 11.9	3.2 ± 0.7	3.5 ± 0.8
Lean strept'n	13	17.0 ± 3.7	2.2 ± 0.1	2.7 ± 0.6
p		N.S.	N.S.	N.S.
Obese control	13	618.0 ± 100.0	24.0 ± 1.8	32.5 ± 5.5
Obese strept'n	13	36.0 ± 7.5	5.5 ± 2.2	0.9 ± 0.4
p		<0.001	<0.001	<0.001

6 animals in each group. Lipogenesis was measured 8 days after streptozotocin treatment. Lean and obese control data identical to Table 3.

x - perimetrial fat;   xx - subcutaneous fat.

^{a} $p < 0.01$ compared to age matched lean control.
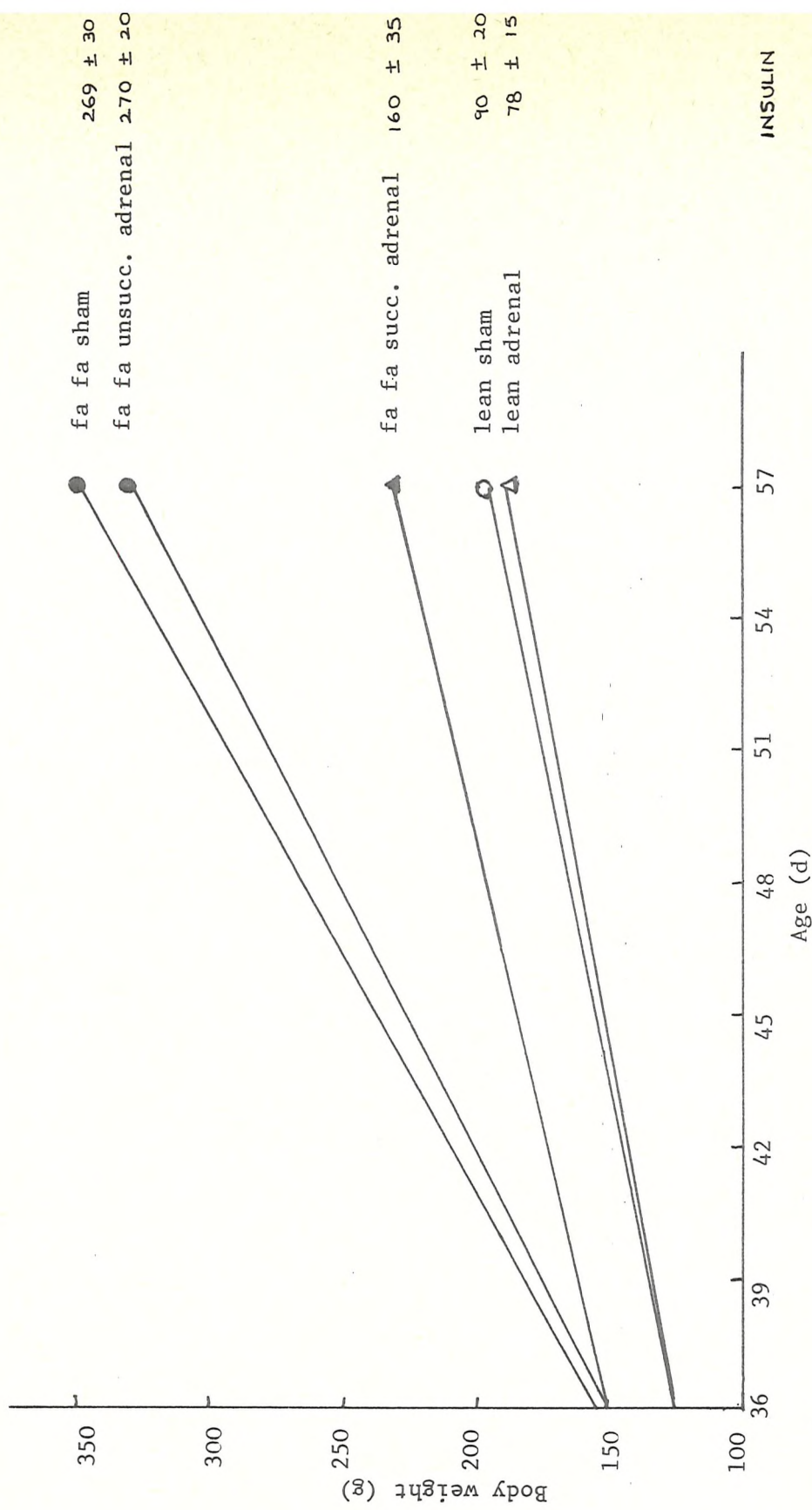
Fig. 6. Weight gain and serum insulin levels of fa fa and lean sham operated and adrenalectomised rats.

INSULIN

fa fa sham	269 ± 30
fa fa unsucc. adrenal	270 ± 20
fa fa succ. adrenal	160 ± 35
lean sham	90 ± 20
lean adrenal	78 ± 15

obese rats weighed significantly less compared to their obese sham operated controls. At the time of sacrifice all animals were carefully examined for adrenal tissue. Out of the 12 obese animals operated, four were found to have a small part of one of the adrenal left and all these four continued to gain weight at the same rate as the obese sham operated rats. These four rats were classed as unsuccessfully adrenalecto- mised. In these animals adrenalectomy had little effect on the rates of lipogenesis or on serum insulin levels in the lean rats. Successful adrenalectomy in fatty rats reduced (Table 11) insulin levels towards but not quite to those of lean rats. Lipogenesis in all three tissues was also greatly reduced compared to values of sham operated obese rats. However, lipogenic rates remained higher than values of lean sham and adrenalectomised rats.

Experiments using suckling fatty (pre-obese) rats

The principle abnormalities of the obese fatty rat are hyperphagia, hyperinsulinaemia and excessive lipogenesis mainly of hepatic origin. These changes are related to the deposition of excess fat. However, it does not follow that any one of these represents the primary lesion. The major problem in identifying the primary genetic defect which should be apparent before the obesity is visually detectable has been the identification of the pre-obese fatty rat.

We have shown that the pre-obese fatty rat can be identified by its lower rectal temperature before these rats can be visually identified as obese. The rectal temperature of pre-obese fatty rats was measured daily from 16 days of age. Of the 51 rats investigated (5 litters) from heterozygote parents, 16 animals showed consistently lower rectal temperature ($34.6 \pm 0.2^{\circ}$C v. $35.4 \pm 0.3^{\circ}$C) and all 16 animals became obese and no animals with the higher normal temperature developed obesity. The temperature profiles of the 5 litters are shown in figure 7. Within each litter there was a clear divergence into two groups on the basis of rectal temperature, although the temperatures were variable between different litters. In addition all male animals had a slightly lower rectal temperature (approximately 0.5°C) than the females in the same litter, but the difference between lean and pre- obese rats was still maintained. Measurement of rectal temperature before day 16 was difficult because of the thermistor probe size.

Table 11   Effect of adrenalectomy on fatty acid synthesis of 8 week old lean and obese rats (Mean ± S.E.)

	LEAN		OBESE	
	Control	Adrenalec'd	Control	Adrenalect'd
FATTY ACIDS (μmole/g/h)				
Hepatic	2.3 ± 0.1	1.9 ± 0.4	18.7 ± 1.9	4.9 ± 2.0[c]
P.M.[x]	1.4 ± 0.3	1.9 ± 0.2	12.9 ± 2.7	5.0 ± 1.9[a]
S.C.[xx]	1.1 ± 0.03	1.1 ± 0.1	15.1 ± 1.9	4.2 ± 1.9[b]
FATTY ACIDS (μmole/whole tissue/hr)				
Hepatic	18.9 ± 1.9	16.8 ± 5.6	217.8 ± 23.4	41.6 ± 2.7*[c]
P.M.	2.6 ± 0.8	2.9 ± 0.1	65.9 ± 14.4	13.4 ± 1.9*[c]
S.C.	3.1 ± 0.7	3.1 ± 0.1	94.0 ± 23.4	20.4 ± 4.0*[b]
SERUM INSULIN (μu/mℓ)				
	90 ± 20	78 ± 15	269 ± 30	160 ± 35
p	N.S.		<0.02	

8 animals in each group were either adrenalectomised or control sham operated at 5 weeks and sacrificed after 3 weeks.

x – perimetrial fat;   xx – subcutaneous fat.

[a] $p < 0.02$;   [b] $p < 0.01$;   [c] $p < 0.001$ – compared to obese control.

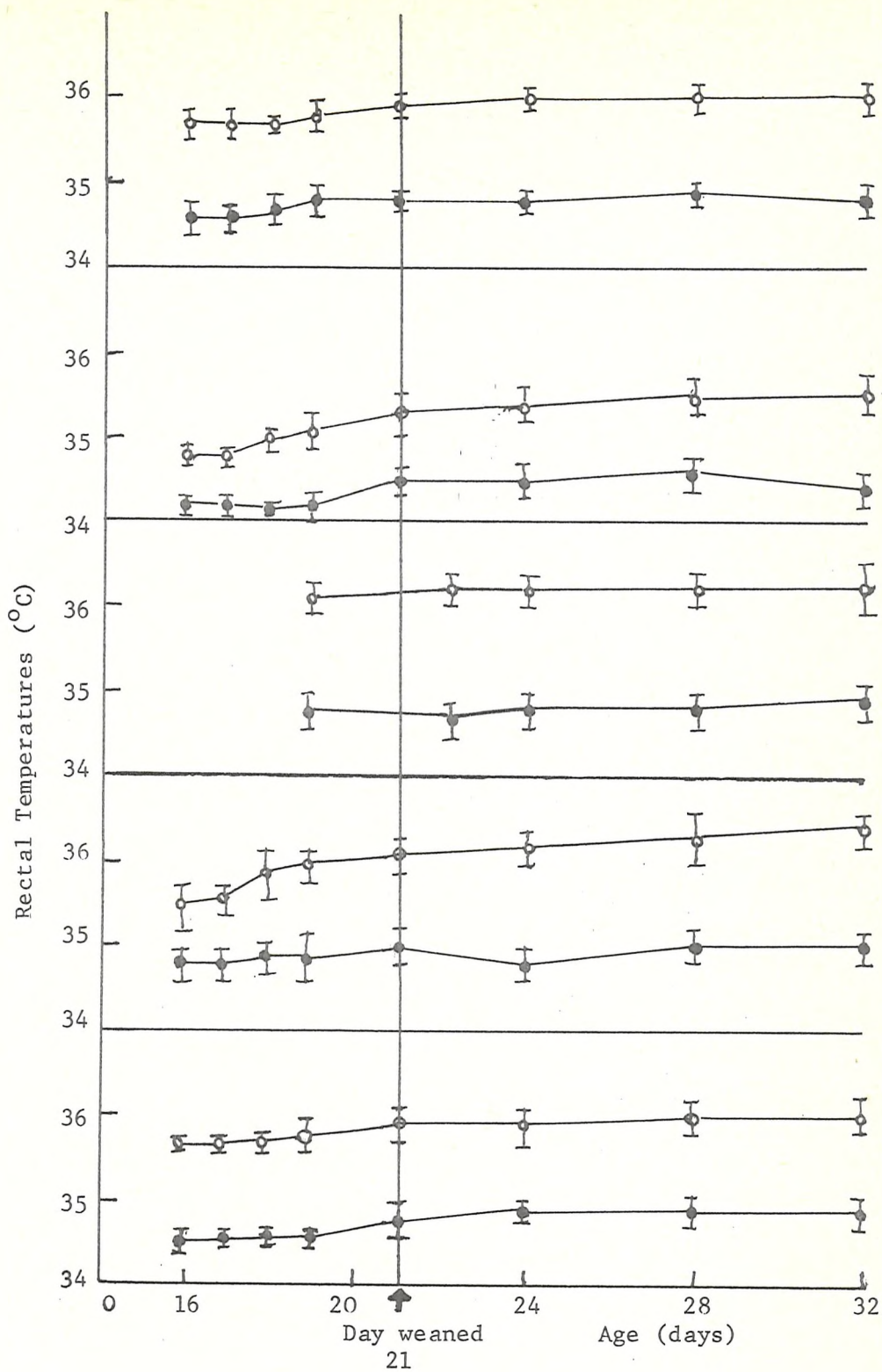* $p < 0.001$ – obese adrenalectomised compared to lean control.

Fig. 7. Rectal temperatures of suckling and weaned (lean (o); obese (●)) rats.

Once established this method was then used for the positive identifi-
cation of pre-obese rats on the basis that the animals should show a low
rectal temperature on three consecutive days (day 16-18). This method of
identification was then adopted for the subsequent studies on hepatic
lipogenesis, G6PDH, ACC and serum insulin in pre-obese fatty rats (Table 12).
However, rectal temperatures on day 18 and 23 only have been included in
the table.

At day 18 (litter A) the depressed rectal temperatures in the pre-
obese fatty rats were again clearly shown. However, at this age hepatic
lipogenesis, insulin levels (Table 12), hepatic G6PDH activity (Table 13),
activity of ACC assayed in the presence and absence of citrate of pre-obese
fatty rats was similar to that of lean rats. In contrast, three days after
weaning at 23 days of age (litter B) it is clear that while the pre-obese
rats still showed a reduced rectal temperature, hepatic fatty acid synthesis
and serum insulin levels showed a three fold increase, with a 190% increased
hepatic G6PDH; 66% increased hepatic ACC in the absence of citrate and
233% hepatic ACC after activation with citrate in the obese rats. The
values of these variables in the fatty rats may be contrasted with a 23 days
old litter born of homozygous dominant parents (Fa/Fa) which contained only
lean offspring (litter C). In such a litter the values of rectal tempera-
ture, hepatic fatty acid synthesis, G6PDH activity and serum insulin were
very similar to those of lean rats in litter B and clearly differed from
pre-obese fatty rats. When weaning was delayed (litter D) 23 days old
suckling animals, previously identified as pre-obese by their low rectal
temperature did not show any increase in hepatic fatty acid synthesis,
G6PDH activity or serum insulin levels.

In contrast, lipogenesis in the subcutaneous adipose tissue (Table 14)
was increased in pre-obese rats from day 10 onwards. The pre-obese pups
could not be identified before the experiment because of the lack of a
smaller rectal probe. In all groups (4 litters in each group) rats born
of heterozygote parents were used and in all the subcutaneous adipose tissue
lipogenesis showed a bimodal pattern of distribution in which approximately
a quarter of the litters showed a three fold increase in adipose tissue
lipogenesis and these same animals had a larger adipocyte size at sacri-
fice, compared to the other animals of the same litter. In contrast the
rate of hepatic fatty acid synthesis in these 10 day old (lean 0.60 $\pm$ 0.10;
pre-obese 0.65 $\pm$ 0.20 μmoles/tissue/hr) and 13 day old (lean 1.1 $\pm$ 0.2;

Table 12   Effect of age and suckling on hepatic lipogenesis and serum insulin levels in lean and preobese rats
(Mean ± S.E.)

AGE (days)	DIET	RECTAL TEMPERATURE (°C) 18	RECTAL TEMPERATURE (°C) 23	FATTY ACIDS (µmole/g/h)	SERUM INSULIN (µu/ml)	NUMBER (n)
A 18 suckling	Fa ?	36.0 ± 0.1	-	0.6 ± 0.1	58 ± 4	45
	fafa	34.1 ± 0.1	-	0.6 ± 0.1	64 ± 2	11
B 23 weaned onto chow	Fa ?	36.5 ± 0.1	36.6 ± 0.2	5.4 ± 0.7	54 ± 3	28
	fa fa	35.0 ± 0.2	35.2 ± 0.2	25.2 ± 2.0[a]	185 ± 13[a]	10
C 23 weaned onto chow	Fa Fa	-	36.8 ± 0.5	2.9 ± 0.3	57 ± 4	8
	Fa ?	36.7 ± 0.2	36.5 ± 0.1	1.8 ± 0.2	54 ± 3	25
D 23 suckling	fa fa	35.0 ± 0.1	35.3 ± 0.1	2.3 ± 0.3	59 ± 5	8

Animals in groups B and C were weaned on day 20.

[a] $p < 0.001$ compared to lean rats in same group.

Table 13   Effect of age and suckling on hepatic enzyme activities in lean and preobese rats (Mean ± S.E.)

AGE (days)		DIET	HEPATIC G6PDH (mμmole/mg protein/min)	HEPATIC ACETYL CoA CARBOXYLASE (mμmole/mg protein/min)	
				−citrate	+citrate
A 18	suckling	Fa ?	30 ± 2	1.6 ± 1.0	4.0 ± 1.0
		fa fa	35 ± 3	1.5 ± 0.9	4.0 ± 0.9
B 23	weaned onto chow	Fa ?	38 ± 2	1.8 ± 0.2	6.0 ± 2.0
		fa fa	113 ± 15[b]	13.0 ± 0.8[b]	20.0 ± 9.0[a]
C 23	weaned onto chow	Fa Fa	31 ± 6		
		Fa ?	26 ± 6		
D 23	suckling	fa fa	33 ± 5		

Conditions were as for Table 12.

[a] $p < 0.01$;   [b] $p < 0.001$ compared to lean of same group (paired 't' test).

Table 14   Lipogenesis, Glucose-6-Phosphate Dehydrogenase (G6PDH) and acetyl CoA carboxylase activities in adipose tissue of suckling lean and preobese rats.

	AGE (days)	No.	RECTAL TEMP. (°C)	FATTY ACIDS (μmole/fat pad/h)	CELL SIZE (nℓ)	G6PDH (mμmole/mg protein/min)	ACETYL CoA CARBOXYLASE (mμmole/mg protein/min)	
							− citrate	+ citrate
LEAN	10	28	Not recorded	0.3 ± 0.02	0.07 ± 0.01			
PREOBESE	10	6		2.1 ± 0.20^b	0.15 ± 0.02^b			
LEAN	13	31	Not recorded	0.3 ± 0.02	0.06 ± 0.01			
PREOBESE	13	7		1.3 ± 0.20^b	0.12 ± 0.02^b			
LEAN	18	28	36.0 ± 0.2	0.5 ± 0.03	0.09 ± 0.02	5 ± 2	0.5 ± 0.2	0.8 ± 0.3
PREOBESE	18	7	34.5 ± 0.4	2.1 ± 0.40^b	0.17 ± 0.02^a	100 ± 40^a	2.0 ± 0.4^b	4.0 ± 0.8^b

[a] $p < 0.01$;   [b] $p < 0.001$ compared to lean of same age.

pre-obese 1.0 $\pm$ 0.25 μmoles/tissue/hr) was identical to that in the lean pups of the same age. Only in the 18 day old litters was prior identification possible by low rectal temperatures. These animals also had an increased fat cell size. In this group rats showed a 4.5 fold increase in adipose tissue lipogenesis. This increased lipogenesis is supported by the 20 fold increase in G6PDH and four fold increase in ACC assayed without citrate and five fold increase in ACC assayed after activation with citrate in the pre-obese rats compared to lean littermates.

Enzyme activities could not be obtained in 10 and 13 day litters due to the small amount of subcutaneous fat pads.

Effect of streptozotocin treatment on suckling fatty rats

Pre-obese rats were identified by their lower rectal temperatures and animals were then made diabetic by streptozotocin injection on day 18, returned to the mother and then weaned on day 21. All animals were sacrificed on day 32 when hepatic and adipose tissue lipogenesis was measured.

Streptozotocin treatment of obese rats (Table 15) caused a dramatic decrease in insulin levels to a fifth of levels in obese controls, so that insulin levels were similar to levels of lean controls. Similarly hepatic lipogenesis (per gm tissue and per whole tissue) showed a 13 fold decrease to values which were not significantly different from those of lean controls and diabetic animals. Adipose tissue lipogenesis also fell to values similar to lean controls. However, both adipose tissue sites could not be studied in lean rats and perimetrial tissue of the obese rats could not be studied due to the virtual disappearance of these fat stores in diabetic animals. Even so the Lee Index of diabetic obese rats showed that they remained stunted whereas body weights differed very slightly from lean control rats. The decreased rates of lipogenesis in the obese streptozotocin treated rats are supported by the (Table 16) four fold decrease in hepatic and two fold decrease in adipose tissue G6PDH and hepatic ACC activity compared to obese controls. However, due to the large variability in the obese streptozotocin treated rats, significant differences between lean and obese rats are difficult to draw.

Streptozotocin treatment of lean rats resulted in smaller changes in hepatic lipogenesis and in the recorded enzyme activities.

43.

Table 15 Effect of streptozotocin treatment of suckling rats on lipogenesis of lean and obese rats at 36 days of age.

| RAT | FATTY ACIDS | TISSUE | | | SERUM INSULIN (μu/mℓ) | BODY WT. (g) | LEE INDEX |
		Hepatic	P.M.[x]	S.C.[xx]			
LEAN	μmoles/g/h	3.7 ± 0.9	1.7 ± 0.7	1.0 ± 0.3	86 ± 25	100 ± 10	303
CONTROL	μmoles/tissue/h	16.8 ± 4.9	1.5 ± 0.3	2.0 ± 0.5			
LEAN	μmoles/g/h	1.6 ± 0.3	-	-	39 ± 12[b]	85 ± 15	297
STREPT'N	μmoles/tissue/h	9.3 ± 2.3	-	-			
OBESE	μmoles/g/h	22.8 ± 2.8	8.6 ± 1.3	6.7 ± 0.5	233 ± 42	184 ± 10	413
CONTROL	μmoles/tissue/h	198.0 ± 39.0	33.0 ± 4.7	57.0 ± 3.8			
OBESE	μmoles/g/h	1.7 ± 0.6[a]	-	0.5 ± 0.01[a]	42 ± 17[a]	115 ± 25	367
STREPT'N	μmoles/tissue/h	10.5 ± 1.1[a]	-	1.6 ± 0.2[a]			

8 rats in each group were injected streptozotocin on day 18, weaned on day 21 and sacrificed on day 36.

x - perimetrial fat;   xx - subcutaneous fat.

Lee index = (3√body wt. ÷ nose to anus length) x 1000

a p < 0.001 compared to obese control

b p < 0.01 compared to lean control

Table 16 Effect of streptozotocin treatment of suckling rats on enzyme activities of lean and obese rats at 36 days of age.

RAT	TISSUE	G6PDH (mμmole/mg protein/min)	ACETYL CoA CARBOXYLASE (mμmole/mg protein/min)	
			-citrate	+citrate
Lean control	Hepatic	57 ± 6	0.6 ± 0.2	5.2 ± 1.2
	S.C. [xx]	22 ± 7	2.2 ± 0.3	8.0 ± 0.4
Lean Strept'n	Hepatic	30 ± 3.8[a]	0.5 ± 0.3	4.7 ± 1.0
	S.C.	18 ± 5.0	—	—
Obese control	Hepatic	153 ± 18	3.1 ± 0.4	17.0 ± 2.0
	S.C.	58 ± 15	9.0 ± 1.5	30.0 ± 5.5
Obese strept'n	Hepatic	36 ± 5.3*[d]	1.2 ± 0.6[c]	7.8 ± 2.4[c]
	S.C.	20 ± 7.0[b]	—	—

8 rats in each group were injected streptozotocin on day 18, weaned on day 21 and sacrificed on day 36.

x x - subcutaneous fat.

[a] $p < 0.01$ compared to lean control.

[b] $p < 0.05$; [c] $p < 0.01$; [d] $p < 0.001$ compared to obese control.

*$p < 0.02$ compared to lean control.

Further studies on suckling rats

To try and see if the increased adipose tissue lipogenesis in the pre-obese rats could possibly be due to differences in milk intake between lean and pre-obese pups an assessment of food intake was obtained. The mother was injected with ^3H_2O when pups were 17 days old and the pups then left with the mother for two days before sacrifice. Serum activity of ^3H_2O was recorded hoping that any differences in milk intake between the pups would result in a proportional difference in the label recovered in the serum of pups. No significant difference in specific activity of serum was seen (Table 17) between pups with low rectal temperatures and those with higher rectal temperatures.

In utero studies

To detect possible differences in the synthesis of body lipids at the foetal stage of development, pregnant mothers with a previous breeding history of producing obese litters, were injected with ^3H_2O. One hour later foetuses were quickly dissected from the uterus, weighed and whole body extracted to record d.p.m. in total body lipid and fatty acid fraction recorded. Values are left as d.p.m. recorded because of the impossibility of bleeding the foetuses for serum specific activities of ^3H_2O.

In two of the experiments (Table 18) at which the approximate day of gestation was day 16, two foetuses in each group showed significantly higher values of ^3H incorporation into total body lipids but no differences in ^3H recovered in fatty acids compared to the other foetuses. However, in the third group at day 20 of gestation no differences could be detected between any of the foetuses.

Table 17   Serum specific activity of pups suckling a mother previously injected with ^3H_2O.

AGE (days)	SERUM SPECIFIC ACTIVITY (d.p.m./1000µℓ serum × 10^6)	RECTAL TEMP. ON DAY 18 (°C)
	2.86	35.4
	3.30	35.2
	3.04	34.3
19	3.20	35.5
	2.98	34.0
	3.38	35.1
	3.13	35.0
	3.83	35.8
	3.94	35.6
19	3.68	35.6
	3.78	34.6
	3.88	35.4
	3.89	35.6
	3.62	35.6
	3.59	35.4
19	3.72	35.2
	3.68	34.4
	3.59	35.5
	2.67	34.4
	2.56	35.2
	2.43	35.4
	2.53	35.6
19	2.79	34.4
	2.80	34.2
	2.75	35.7
	2.72	35.7
	2.59	35.5

Mother was injected with 4mCi ^3H_2O when pups were 17 days old and pups left with mother until sacrifice on day 19. Rectal temperatures were recorded on day 17 and day 18.

Underlined values are for pre-obese rats identified by their low rectal temperature.

Table 18   The accumulation of ^{3}H in total body lipids and fatty acids of the foetuses of a mother previously injected with ^{3}H$_2$O.

NUMBER	DAY OF GESTATION	d.p.m. IN TOTAL BODY LIPIDS	d.p.m. IN TOTAL BODY FATTY ACID	WEIGHT OF HEAVIEST PUP (g)	WEIGHT OF LIGHTEST PUP (g)	MOTHER'S SERUM ^{3}H$_2$O ACTIVITY d.p.m./100 μl
8	16	19,985 ± 5,238	7,380 ± 1038	2.7	2.3	1.13 x 10^8
2		38,672 ± 40,698	5,939 ± 6,632			
6	16	24,683 ± 3,310	9,013 ± 927	2.9	2.6	1.93 x 10^8
2		40,132 ± 50,463	7,738 ± 8,650			
11	20	10,859 ± 3,638	3,865 ± 1,689	4.4	4.0	9.36 x 10^6

DISCUSSION

^3H_2O Method for the Estimation of Fatty Acid Synthesis

The ^3H_2O method for the estimation of fatty acid synthesis first prepared by Lowenstein (1971); Windmeuller and Spaeth (1966 and 1967) was used in these reported studies. This method has distinct advantages over the use of ^{14}C labelled substrates in that it overcomes any difficulties in substrate pool sizes that may be obvious in comparing two such different animals lean and obese rats. The specific activity of ^3H_2O pool is measured on a sample of serum. However, this method does assume that the tissue water specific activity and serum water specific activity are identical. Our experiments indicate that tissue water specific activity does not finally attain serum water specific activity until nearly one hour although there was a very rapid initial equilibrium up to 75% specific activity. This delay in final complete equilibration introduces a small error in the calculation of fatty acid synthesis but since the time course of tissue water specific activity in lean and obese rats were very similar the comparative rates of lipogenesis are valid as calculated.

The rates of fatty acid synthesis were very similar to other published values. The ^3H_2O method is now widely used for both in situ and in vitro experiments and the published rates of lipogenesis vary from 5 μmoles/hr in vivo studies (Lowenstein, 1971) to 13.3 moles in vitro experiments (Windmeuller and Spaeth, 1967) in rats to 1-7 μmoles fatty acids per gram in the perfused liver of mice (Salmon et al, 1974).

Fatty acid synthesis in the obese rat

The genetically obese rat is characterised by excess deposition of fat in both the subcutaneous and intra-abdominal fat depots. The increased fat stores are accommodated by the increase in both number and size of fat cells (Johnson et al, 1971). The majority of studies on lipogenesis in the obese rat have been confined to animals older than two months in age (Zucker, 1965; York and Bray, 1973b) when the obesity is already profound. The following sections will concentrate on the effects of age and lipogenesis on the distribution of total lipogenic capacity between liver and adipose tissue and upon the mechanisms underlying the hyperlipogenesis of the obese rat.

At five weeks of age the rate of fatty acid synthesis per gram tissue was greatly enhanced in both the liver and the adipose tissue of obese rats whereas at thirteen weeks the hepatic lipogenesis had increased further, whereas the adipose tissue lipogenesis had decreased to values close to those observed in lean rats. The interpretation of this data is complicated by the increase in both liver and adipose tissue weights of obese rats such that the total fatty acid synthesis in liver of obese rats is increased still further. The fall in adipose tissue, fatty acid synthesis per gram tissue in thirteen week obese rats is not an artifact of increased fat cell size since total synthesis of fatty acids by both subcutaneous and perimetrial fat pads falls to levels similar to thirteen week lean rats. Despite the large increase in adipose tissue mass the fall in fatty acid synthesis in the adipose tissue occurs despite an increase in insulin levels. Similar findings have been reported from in vitro experiments. York and Bray (1973) demonstrated a twenty to thirty fold increase in fatty acid synthesis of adipose tissue sites from six week old obese rats in cells which were still very sensitive to insulin stimulation. In contrast in older obese rats (17 weeks) fatty acid synthesis in the adipose tissue had fallen to lean levels and the adipose tissue was insensitive to insulin stimulation (York and Bray, 1973b; Martin and Lamprey, 1975; Zucker and Antoniades, 1972). The fall in adipose tissue lipogenesis with age is likely to be a result of developing insulin insensitivity whereas the increasing hepatic lipogenesis parallels the rise in serum insulin.

All rates of lipogenesis in lean and obese rats were measured between 10 a.m. and 11 a.m. and since the rat is a nocturnal animal it was necessary to follow the diurnal variation. From this available data it is possible to calculate approximately the value for the total daily synthesis of fatty acids in these rats making the following assumptions.
(1) The perimetrial and posterior subcutaneous fat pads represent 50% of the total adipose organs.
(2) Other fat organs like the scapular and the retroperitoneal have similar rates of lipogenesis.
(3) The rate of lipogenesis was constant for each six hour period of the diurnal rhythm.
(4) The average chain length of synthesised fatty acid was 16 carbons.

In five week old lean rats the calculated daily synthesis of fat amounts to 763 μ moles/ day ($\backsim$ 0.2g Triglyceride) and in obese rats 9018 μ moles/day ($\backsim$ 2.5g Triglyceride) assuming a molecular weight of 256. Zucker (1975) in detailed studies of body composi-

tional changes has shown that the obese rat fed ad libitum may deposit up to 2.7g of fat per day at this age whereas a lean rat will deposit fat only at the rate of 0.4g/day. Bray et al (1973) have reported fat deposition in older 17 week obese rats at about 1.5g/day. Thus the calculated values which do not allow for the catabolism of newly synthesised fatty acids agree well with the rate of growth of the obese rat.

Relative importance of the liver and the adipose tissue

Both the liver and adipose tissue are capable of synthesising fatty acids from endogenous substrates. However, the relative importance of each tissue varies with animal species. Thus whereas in the mouse the liver is thought to be the major site for fatty acid synthesis (Hems et al, 1975), in the obese ob ob mouse the major proportion of fat synthesised is located in the adipose tissue. Although only a selected number of adipose depots were investigated in the reported experiments the evidence would indicate that the liver is also the major site of fatty acid synthesis in the lean rat. Thus on the assumption that the fat depots investigated represented approximately one half of the total discrete adipose tissue stores the ratio of lipogenesis in the liver to adipose tissue (L:AT) in lean rats at 10.00 hours was 3.5 at 5 weeks and 3.0 at 13 weeks. Since there was little transfer of ^{3}H fatty acid from the liver to adipose tissue during the time of the experiments (Table 4 and fig. 3) these L:AT ratios are indicative of the predominant role that the liver plays in fatty acid synthesis in the lean rat. A similar conclusion has been reported for the lean mouse (Hems et al, 1975) but not the obese ob ob mouse.

These reported studies also suggest that the liver is primarily responsible for the excess fatty acid synthesis in both young (5 week) and mature (13 week) obese rats. This conclusion can be drawn from the following observations. Although the L:AT ratio for fatty acid synthesis at 10.00 hours in 5 week obese rats was only 2.0 (approximately 1.0 if it is assumed that only 50% of the adipose stores were analysed), the experiment with hepatectomised rats (Table 4) indicate that the majority of the adipose tissue ^{3}H fatty acid had initially been synthesised in the liver or possibly the intestine. It is possible that the low accumulation of ^{3}H fatty acid in adipose tissue of obese rats after hepatectomy could partly be due to the fall in serum insulin and glucose levels

during the experiment. However, similar falls in these variables in lean rats did not affect adipose tissue fatty acid synthesis during the one hour time course of the experiment. The importance of the hepatic contribution to adipose tissue fatty acid synthesis was supported by the very rapid appearance of ^{3}H fatty acid in serum triglyceride in obese rats and by the fall in half life of serum triglyceride. After addition of this transported ^{3}H fatty acid fraction to the hepatic ^{3}H fatty acid, the L:AT ratio for fatty acid synthesis in 5 week obese rat rises to 26. In quantitative terms, this represents a nine fold increase in total hepatic lipogenesis in obese rats as a result of both the increased rate (per gram) and the increase in liver size. Indeed it can be calculated that the rate of turnover of serum triglyceride was increased from O.8 mg/min in lean rats to 2.8 mg/min in obese rats. A similar increased turnover rate for triacylglycerol in the obese rats (5 mg/min) compared to lean rats (2 mg/min) using labelled thoracic duct chylomicrons is reported (Redgrave, 1977). The increased synthesis of hepatic fatty acids and increased turnover of serum triglyceride are consistent with the fundamental role of the liver in the hyperlipogenic state of the obese rat. The early observation of Barry and Bray (1969) had suggested that much of the hypertriglycerideaemia was of hepatic rather than intestinal origin. In addition the studies of Schonfeld and Pfleger (1971) and Schonfeld et al (1974) with perfused liver preparations show the increased secretion of very low density lipoproteins (VLDL) from the livers of obese rats. This VLDL was of normal structure but contained a higher proportion of triglycerides. In our experiments the time course for labelling of serum triglycerides in obese rats was very rapid and much faster than is normally observed in lean animals, thus indicating the enhanced potential for VLDL secretion. Although the adipose tissue lipoprotein lipase activity is increased in the obese rat (Gasquet et al, 1973) serum triglycerides remain elevated. This presumably results from the enhanced production and secretion of VLDL. Thus the fatty rat clearly differs from the obese ob ob mouse in which the adipose tissue is responsible for the major proportion of the excess lipogenesis. (Hems et al, 1975).

Despite the dominance of hepatic lipogenesis, fatty acid synthesis in adipose tissue of obese rats was also increased in young animals in confirmation of previous in vitro experimental reports (Bray et al, 1970; York and Bray, 1973; York and Bray, 1973b,

Martin and Lamprey, 1975). The early increase in adipose tissue fatty acid synthesis is probably a result of the hyperinsulinaemia but this is followed by the development of its resistance as previously discussed. In contrast no such insensitivity was observed in the liver. Indeed the lipogenic pathways in the liver of the obese rat appear to be under maximum insulin stimulation. A similar picture has also been described for the obese ob ob mouse (Assimacapoulos J and Jeanrenaud, 1976).

In the 5 week obese rats the enzyme profile of the liver and adipose tissue Glucose 6 phosphate dehydrogenase and acetyl CoA-carboxylase reflect the increased hepatic and adipose tissue lipogenesis compared to lean 5 week old rats. The increase in activity of acetyl CoA carboxylase in the presence of citrate suggests that there is also more enzyme protein in the obese rats. Other studies have shown that the majority of enzymes associated with lipogenesis, e.g., acetyl CoA carboxylase, ATP citrate lyase, malic enzyme, 6 phosphogluconate dehydrogenase, glycolytic enzymes (glucokinase and pyruvate kinase) and gluconeogenic enzymes (glucose 6 phosphatase and fructose 1-6-diphosphatase) are increased in young (4-8 week) obese rats. (Taketomi et al, 1975; Martin, 1974; Martin and Lamprey, 1975).

Dietary effects on lipogenesis in the obese rat

Fatty acid synthesis in the liver and adipose tissue is responsive to both the composition of diet and the quantity of food eaten. In particular sucrose feeding has been shown to increase lipogenesis, its main effects being located in the liver (Mack et al, 1975). The increased utilization of sucrose by the liver has been associated with the presence of two enzymes capable of metabolizing fructose, fructokinase (E.C. 2.7.1.4) and fructoaldolase (E.C.4.1.2.b). We investigated the response of obese rats to sucrose feeding in order to ascertain if the control of lipogenesis in obese rats was normal with respect to fructose metabolism. Previous evidence from experiments with isolated hepatocytes had shown that the liver of obese rats had a normal substrate profile for fatty acid synthesis (Bloxham et al, 1977) that is lactate and pyruvate are good carbon sources for fatty acid synthesis but glucose carbon is poorly incorporated into fatty acids.

The stimulation of hepatic fatty acid synthesis on sucrose feeding was clearly shown in the lean rats, it was associated with an increase in serum insulin and in the activity of hepatic glucose-6-phosphate dehydrogenase (other lipogenic enzymes were not assayed). In contrast, neither fructokinase nor fructoaldolase showed any increase in

activity after sucrose feeding. These results suggest that the activity of these enzymes are normally sufficient to produce an increased substrate supply (Acetyl CoA) for lipogenesis and that other rate limiting processes for example, pyruvate dehydrogenase or acetyl-CoA-carboxylase, were probably activated under the influence of enhanced insulin stimulation (Coore et al, 1971; Weiss et al, 1971). There was also an enhanced accumulation of fatty acids in the adipose tissue of lean sucrose fed animals. This could reflect either an hepatic contribution or an increased metabolism of fructose by the glycolytic enzymes of the adipose tissue. This latter explanation seems unlikely because of an unfavourable km for fructose by hexokinase (1.5) in comparison with the km for glucose (0.15). A similar increase in lipogenesis was seen in sucrose fed obese animals although in this case the enhanced accumulation of fatty acid was only seen in the liver, this could be interpreted as indicating that hepatic VLDL secretion was already at a maximal rate in the chow fed obese rats.

The obese rat is hyperphagic, its food intake being increased by up to 25 to 30% at 6 weeks of age. However, a number of studies which have investigated the effects of reduced food intake have shown that the obese rat still develops a moderate obesity when its food intake is restricted to that of lean rats. In order to investigate the effects of pair feeding on fatty acid synthesis it was necessary to adapt both lean and obese to a meal feeding pattern. Without this control the lean animal would continue to eat many small meals during the day whereas the obese rat would eat all of its reduced food intake in a short period. Such a gorging meal pattern has been shown to result in the induction of a hyperlipogenic state (Lowenstein, 1971; Margot et al, 1977). This was clearly observed when lean animals were fed on this meal feeding pattern, fatty acid synthesis increased in both the liver and the adipose tissue compared to ad libitum fed nibbling lean rats and this was associated with the increased serum insulin levels. In contrast, when food intake of obese rats was restricted to lean levels serum insulin levels fell and were no longer different from meal fed lean rats and in addition the synthesis of fatty acids per gram fell close to meal fed lean animals although the total tissue lipogenesis remained higher due to the increased liver and adipose tissue mass in the obese animals.

However, these results differ from Martin's (Martin, 1974) who found that hepatic lipogenesis of pair fed obese rats was twice that of lean controls. The explanation of this may reside either in his use of much older obese rats that were pair fed for a longer

time, the use of ^{14}C acetate as a marker for lipogenesis or the higher insulin levels in the pair fed obese (274 $\pm$ 56 μu/ml) compared to the lean (53 $\pm$ 6 μu/ml) rats (Martin and Gahagan, 1977).

When lean rats were fed a high sucrose diet on the same meal feeding regime the rate of synthesis of fatty acids in liver and adipose tissue increased still further and approached those seen in obese rats. Serum insulin levels were identical in both lean and obese animals under these conditions. These studies suggest that the increase in serum insulin, observed in obese rats was possibly responsible for the hyperlipogenesis since under suitable conditions the rate of fatty acid synthesis in lean approached the high levels seen in obese rats while fatty acid synthesis in the obese was reduced towards normal lean values when insulin was decreased by pair feeding and food restriction.

Factors controlling fatty acid synthesis in the fatty rat
(I) Adrenal corticosteroids

Although the obese rat is characterised by hypertrophy of adrenal cortex (Bray and York, 1971b) there is some conflict over the status of serum adrenal steroid concentration. Although Yukimura et al (1978) could not demonstrate any changes in serum corticosterone or in its diurnal variation (Martin et al, 1978) found that serum corticosterone was increased during certain periods of the day. Adrenalectomy has been shown to reduce insulin and blood glucose of the obese ob ob mouse (Solomon and Mayer, 1973) although it does not prevent the obesity. However, after adrenalectomy of obese rats (Yukimura et al, 1978) there was a normalisation of food intake and fall in insulin level and an increase in bone growth. In addition there was no further increase in the percentage of body fat after adrenalectomy. In the reported experiments adrenalectomy resulted in the lowering of hepatic and adipose tissue fatty acid synthesis and serum insulin levels compared to obese sham operated controls. Adrenalectomy did not seem to affect hepatic or adipose tissue lipogenesis or insulin levels in lean rats. On examining the obese adrenalectomised rats at the time of sacrifice we noticed that even if the slightest bit of adrenal was left at the time of operation or regenerated during the two week post operative period, this was sufficient to maintain the normal weight gain as well as the normal high rates of lipogenesis and insulin levels observed in obese sham operated controls which had

both adrenals intact. In adrenalectomised obese rats weight gain paralleled the weight gain of lean rats but lipogenesis and insulin levels remained higher than those observed in lean controls. These results are consistent with the observations of Yukimura et al, 1978) since the maintenance of an identical weight gain to lean rats whilst maintaining a constant but higher percentage body fat composition necessitates a higher deposition in the obese compared to adrenalectomised lean rats. These results also suggest that the inhibitory effects of hypophysectomy on the development of obesity in obese rats (Powley and Morton, 1976) were mediated through the inhibition or loss of adrenal glucocorticoids.

The actions of adrenal steroids on fatty acid synthesis are complex. Adrenalectomy has been shown to result in the enhancement of adipose tissue fatty acid synthesis and in the induction of fatty acid synthetase when adrenalectomised animals are pair fed to their operated controls (Volpe and Marasa, 1975). However, as clearly shown in the reported experiments, adrenalectomy of obese rats resulted in a fall in serum insulin and also in food intake (Yukimura et al, 1978). Thus it would appear that the indirect effects of adrenal corticosteroids predominate over the direct effects on adipose tissue lipogenic enzymes. In addition, the evidence again points to the relationship of serum insulin levels to the rate of fatty acid synthesis in the obese rats.

(2) Insulin

Obesity is associated with the hypersecretion of insulin as well as induced secretion by glucose and other stimuli. Increase in insulin level increases lipogenesis from various substrates, activity of lipogenic enzymes, esterification of fatty acids to triglycerides, and a decrease in oxidation of fatty acids to ketone bodies. All these changes favour the increase in triglyceride synthesis, with resulting intracellular accumulation of lipids and the augmentation of the secretion of triglycerides as VLDL. The important role of hyperinsulinemia in producing these changes is illustrated in obese ob ob mice by the reversal of these changes towards normal upon decreasing hyperinsulinemia (Assimacopoulos - Jeannet et al, 1974; Loten, et al, 1974).

To study the role of hyperinsulinemia in the development and the maintenance of obesity in these Zucker rats, diabetes was induced in suckling, weaned young (4 weeks) and weaned older (12 weeks) rats, by streptozotocin injection. Streptozotocin treatment of weaned obese (4 weeks and 12 weeks) animals resulted in the lowering of hepatic,

perimetrial and subcutaneous fatty acid synthesis and insulin levels to those of lean values. However, the total adipose tissue lipogenesis in diabetic 4 week old rats was higher than lean values due to the already increased tissue mass. However, streptozotocin treatment of suckling animals resulted in lipogenesis per gram or total hepatic, perimetrial and subcutaneous fat pad adipose tissue to similar values noted in lean controls. Similarly, insulin levels and enzyme activities were all reduced. These results show that if insulin levels in the obese rats are kept suppressed before weaning then not only are the rates of lipogenesis reduced but also tissue weights are reduced, thus insulin plays an important role in the development as well as the maintenance of obesity.

Developmental changes in the suckling pre-obese rats

The obese rats are bred from heterozygotes so that approximately a fourth of the litter are obese. However, they cannot be visually detected as obese until about 4 weeks of age. The reported anomalies detected at the level of either the liver, the adipose tissue, adrenals, hypothalamus or the pancreatic β cell have been reported in weaned visually obese rats so that the fundamental error which is responsible for the genetic lesion in the fatty rat is not understood. The investigation of the pre-obese stage has been hampered by the lack of a simple diagnostic method for differentiating between the lean and pre-obese rats. We have demonstrated a simple method, the measurement of rectal hypothermia which may be used to identify obese rats prior to overt obesity. Every animal that became obese had a lower rectal temperature and there were no exceptions to these observations. These findings are in agreement with those of Kaplan (1977) who showed a reduction of oxygen consumption in the pre-obese fatty rat. These are also similar to those reported in the pre-obese ob ob mouse (Kaplan and Leveille 1974; Trayhurn et al, 1977).

During the preweaning period the pre-obese rats are characterized by an increase in carcass lipid content by 13 days of age (Johnson et al, 1971; Bell and Stern, 1977) and this is supported by the finding that pre-obese rats show an enlargement of the adipose tissue cells (Boulange et al, 1978) by 5-7 days of age. Our studies too show an increased adipocyte size by day 10 (youngest day studied) in pre-obese rats. However, this increase in carcass lipid content of pre-obese rats cannot be explained

by an increase in hepatic lipogenesis, the accompanying induction of glucose-6-phosphate dehydrogenase, acetyl CoA carboxylase or insulin levels because our studies show that all these factors are normal in the suckling pre-obese animals and do not appear until after weaning. On postponing the weaning of these animals we showed a postponement in the increase in all the above mentioned factors. Increased milk intake also cannot explain this increased lipid deposition. Radioactivity measured in the plasma of suckling pups drinking ad libitum tritiated mothers milk show no difference between the lean and pre-obese pups and these results are similar to the reported studies from water turn-over determinations (Boulange et al, 1978b). The normal hepatic lipogenesis, glucose-6-phosphate dehydrogenase and insulin levels of the pre-obese rat may be related to the high fat diet provided to the suckling pups by the mother's milk, a diet that would provide little substrate for lipogenesis as well as keep the hyper-insulinemia of the pre-obese fatty rats at moderate levels. Weaning thus appears to be an important regulatory signal in the development of obesity in the obese rat and could well be associated with the change in diet from a high fat diet to a high carbohydrate chow diet at weaning.

The excess fat deposits that have been shown at weaning in the obese rats by analysis of body composition must result from the increased adipose tissue lipogenesis, increased adipose tissue glucose-6-phosphate dehydrogenase and acetyl CoA carboxylase activities that we have reported in suckling pre-obese rats from day 10 onwards when hepatic lipogenesis, insulin levels and food intake are normal. At this stage no differences in activity between the two types is reported; however, energy expenditure shown by lower oxygen consumption (Kaplan, 1977) and lower rectal temperature along with the reported increase in adipose tissue lipoprotein lipase (Boulange et al, 1978b) could lead to the increased fat deposits observed in the pre-obese rats.

From the results it is clear that hypothermia and metabolic changes in the adipose tissue could well be linked with the area of metabolism which could be responsible for the genetic fault and these definitely seem to appear even before hypoactivity, hyper-insulinemia, increased hepatic lipogenesis and hyperphagia. The increase in adipose tissue lipogenesis when hepatic lipogenesis, insulin levels and food intake are all normal

A Schematic Diagram of Genetic Obesity

Sterility

Impaired gonadotrophic production

ENZYME DEFECT

HYPOTHALAMUS

↑ACTH

↑Adrenal steroids

β cell

↑INSULIN

INSULIN RESISTANCE

OTHER TISSUES

↓ glucose uptake muscle

↑Food intake

↑LIPOGENESIS

↑FAT DEPOSITS

OBESITY

↓Thermogenesis

↑Calorific Efficiency

↓Activity
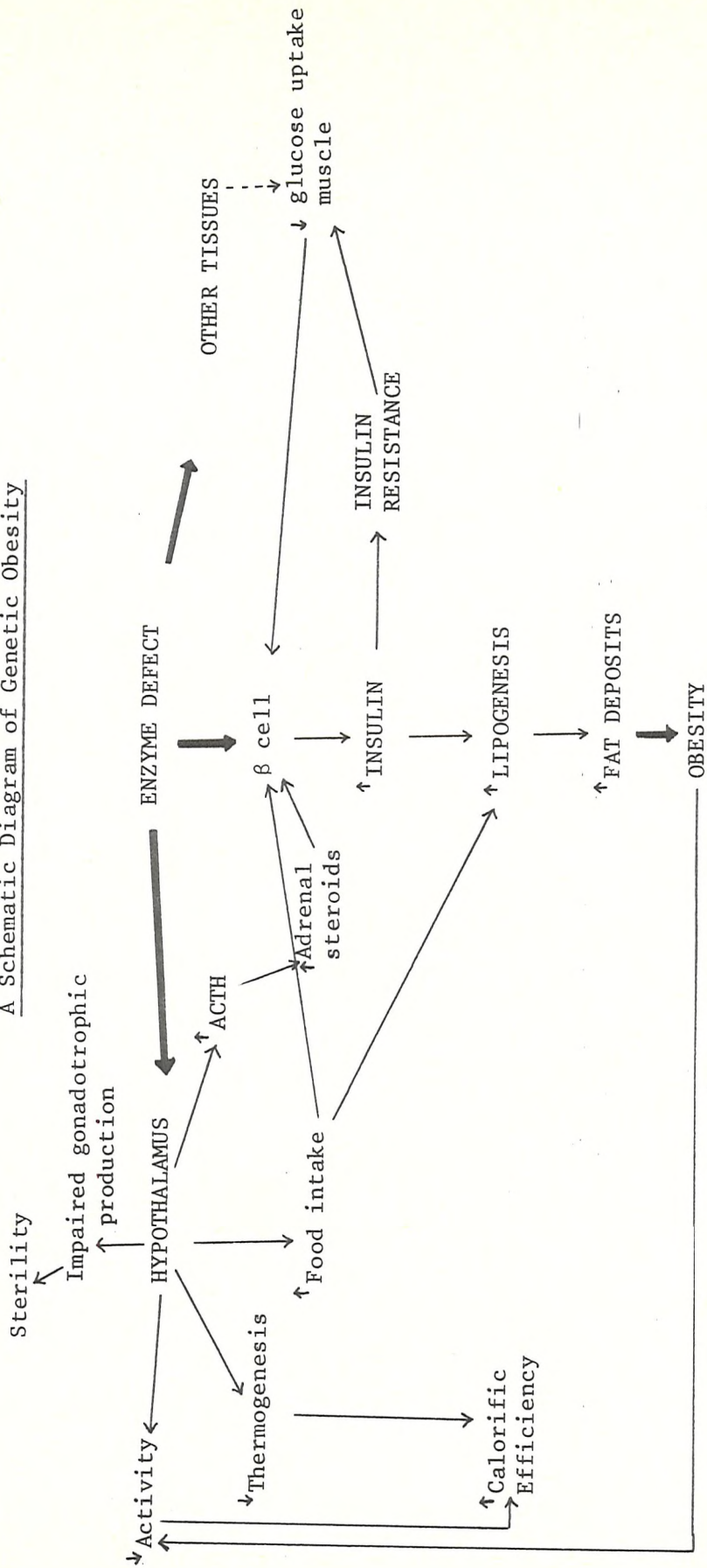
is difficult to understand. However, acetyl CoA carboxylase measurements show that the enzyme protein is increased though the cause for this increase is not known.

This recessively inherited form of obesity represents a single molecular change in the DNA sequence for some peptide which may be either an enzyme or a structural protein. It is likely that the genetic defect may be expressed independently at various sites that could in turn affect their metabolic processes, all resulting finally in increased lipogenesis and therefore obesity. In the obese ob ob mouse (York et al, 1978) the thyroid dependant ($Na^+ + K^+$) ATP ase has been pointed to be defective in a number of tissues of these animals and so could explain these syndromes of obesity. It is very possible that another such widely distributed enzyme is likely to form the basis of the genetic lesion in the obese rat.

The figure (York - Genetic models of obesity in laboratory animals - to be published in 1979) presents a possible layout for the types of interaction which might be involved in the animal obesities. A defective enzyme could lead to a wide ranging effect leading to secondary changes that are common to this model of obesity. Since the enzyme activity of the thyroid dependant ($Na^+ + K^+$) ATPase is normal in the obese rat, the look out for some other such enzyme has to continue.

Summary

From the results obtained, the following conclusions can be drawn.

(1) The liver is the major site of fatty acid synthesis in the rat.

(2) Hepatic and adipose tissue lipogenesis of 6 week old obese rats is increased.

(3) The majority of excess fatty acid synthesis in the obese rat is in the liver.

(4) Calculated rates of lipogenesis agree with observed rates of weight gain.

(5) The hyperlipogenesis of obese rats after weaning is secondary to the hyper-insulinemia, hyperphagia and increased adrenal corticosteroid secretion.

(6) Under suitable conditions of increased insulin levels lipogenesis in the lean rats can be augmented to reach values observed in the obese rats.

(7) Pre-obese suckling obese rats may be identified by their low rectal temperatures.

(8) Hypothermia precedes increased rates of hepatic lipogenesis, induction of glucose-6-phosphate dehydrogenase and increased plasma insulin in the obese rats.

(9) Weaning plays an important role in the increase in hepatic lipogenesis, glucose-6-phosphate dehydrogenase, acetyl CoA carboxylase and insulin levels in the obese rats.

(10) Adipose tissue lipogenesis and adipocyte size are increased in pre-obese suckling obese rats by day 11 before an increase in insulin levels and hepatic lipogenesis.

(11) Hypothermia and metabolic changes in the adipose tissue could well be closely linked with the primary genetic defect in the obese fatty rat.

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