PATHOGENESIS OF THE OBESE-HYPERGLYCEMIC

SYNDROME IN MICE (GENOTYPE $\frac{ob}{ob}$)

H.F.P. JOOSTEN

ERRATA

page 48, 11th line from the bottom :
for "standerdized" read "standardized".

page 50, 4th line from the bottom :
for "3   thyroxin" read "3 μg thyroxin".

page 56, 4th line from the top :
for "hypothroidism" read "hypothyroidism".

page 59, 2nd line from the top :
for "Lissretation" read "Dissertation".

page 88, 3rd line from the bottom :
for "for h" read "for her".

page 93, 4th line from the top :
for "lates" read "latest".

page 94, 17th line from the top :
for "abtained" read "obtained".

page 96, 12th line from the top :
for "avout" read "about".

page 97, 14th and 15th line from the top :
for "couls" read "could" and for "tissue-immnumological" read
"tissue-immunological".

PATHOGENESIS OF THE OBESE-HYPERGLYCEMIC SYNDROME IN MICE (GENOTYPE $\frac{ob}{ob}$)

Promotor:
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PATHOGENESIS OF THE OBESE-HYPERGLYCEMIC

SYNDROME IN MICE (GENOTYPE $\frac{ob}{ob}$)

PROEFSCHRIFT

TER VERKRIJGING VAN DE GRAAD VAN DOCTOR IN DE
WISKUNDE EN NATUURWETENSCHAPPEN AAN DE KATHO-
LIEKE UNIVERSITEIT TE NIJMEGEN, OP GEZAG VAN DE
RECTOR MAGNIFICUS PROF. Mr F.J.F.M. DUYNSTEE
VOLGENS BESLUIT VAN HET COLLEGE VAN DECANEN
IN HET OPENBAAR TE VERDEDIGEN OP DONDERDAG
25 APRIL 1974 DES NAMIDDAGS TE 4 UUR

DOOR

HENRICUS FRANCISCUS PETRUS JOOSTEN

GEBOREN TE HORST

aan vader en moeder
Riny

The present dissertation is based on the following papers :

I.   Harrie F.P. Joosten and Piet H.W. van der Kroon :
     The enlargement of epididymal adipocytes in relation
     to hyperinsulinemia in obese–hyperglycemic mice $(\frac{ob}{ob})$.
     Metabolism 23, 59, 1974.

II. Harrie F.P. Joosten and Piet H.W. van der Kroon :
     The role of the thyroid in the development of the
     obese–hyperglycemic syndrome in mice $(\frac{ob}{ob})$.
     Accepted for publication in Metabolism.

III. Harrie F.P. Joosten and Piet H.W. van der Kroon :
     Growth pattern and behavioral traits associated with
     the development of the obese–hyperglycemic syndrome
     in mice $(\frac{ob}{ob})$.
     Submitted to Metabolism.

IV. Harrie F.P. Joosten, Piet H.W. van der Kroon and
     Anton J.M. Buis :
     Development of the obese–hyperglycemic syndrome in
     mice with a growth hormone deficiency.
     Submitted to Metabolism.

CONTENTS

1. General objective of the study of the obese-hyperglycemic
    syndrome in mice $(\frac{ob}{ob})$.

The ultimate aim of this study was the definition of the primary
genetic defect in homozygous obese mice by performing a tho-
rough investigation of the pathogenesis of the obese-hypergly-
cemic syndrome in these mice. The results of this study might
be relevant in regard of a better understanding of the causes
of diabetes and obesity in man.

Diabetes and obesity are diseases with a high prevalence,
especially in our prosperous, western part of the world (1).
Besides genetic factors, environmental factors such as the
type of diet and eating habits play an important role in the
induction of diabetes and obesity. It appears to be difficult
to choose the right diet with respect to both, quality and
quantity. While large parts of the third world are suffering
from undernutrition and/or the use of imbalanced diets, the
rich, western part of the world experiences the disadvantages
of overnutrition.

Each of the two diseases, diabetes and obesity influences the
rate of morbidity and mortality (2, 3, 4, 5). The obese diabetic
must face even higher risks.

In obesity, the predominant adverse effect appears to be on
the cardiovascular system (1, 2). Overweight persons have higher
risks for hypertension, atherosclerosis and diabetes. The posi-
tive correlation between overweight and mortality has been
clearly revealed by the statistics of insurance companies in
the United States. The greater the degree of overweight, the
greater the risk of death. The number of overweight persons
dying from cardiovascular or renal diseases is increased as com-

pared to normal weight persons with a factor of 1.50 to 1.75.
The risk to die from diabetes is increased with a factor of
4 in overweight persons.

The American Diabetic Association estimates that one of twenty
persons has diabetes, or is potentially a diabetic person (1).
About two-thirds of the cases of diabetes occur after fourty
years of age. Diabetics have a higher risk for some diseases
such as, nephropathy, neuropathy, retinopathy and vascular dis-
eases (3, 4, 5). In Great Britain, diabetic retinopathy is the
principal cause of newly occurring blindness (5).

In persons developing diabetes after maturation there is a high
incidence of obesity ; diabetic persons are often obese. On
the other hand, the percentage of obese individuals developing
diabetes is small (1). There is no clear explanation for the
frequent association of diabetes and obesity. A number of me-
tabolic anomalies is commonly found to be associated with both
diseases. Examples such as a certain hyperinsulinemia and ab-
normalities in the insulin secretion kinetics of the islands
of Langerhans following stimulation by carbohydrates, are well
known.

In addition to a good control of the dietary intake of carbo-
hydrates, several drugs can be used which either improve the
island function or insulin sensitivity. In severe cases, exo-
geneous insulin can be administered to replace or support the
patients own insulin. Therapeutical means to treat diabetes
are manifold.

So far however, means to prevent diabetes and obesity are
either lacking, or very limited. Until today, it is virtu-
ally impossible to prevent the development of diabetes and
obesity, in particular if these diseases are determined geneti-
cally. This situation will persist as long as the etiology of

these diseases is not completely understood. Considering tne
high incidence of obesity and diabetes in man, and its econo-
mical, psychological and sociological implications, it seemed
appropriate to perform some fundamental studies on the etiolo-
gy of these diseases as a contribution to a better understan-
ding of the primary causes in particular in genetically deter-
mined diabetes and obesity. Since the human patients are not
the most suitable objects for experimental research, a study
of diabetes and obesity was performed on mice.

Although for the study of diabetes and obesity, several labora-
tory animals are suitable (6, 7, 8), only those belonging to
the genus Mus musculus (house mouse) are listed below.

I. Mice with genetically determined diabetes and/or obesity.

monogenic	dominant	A^y	yellow (9)
		A^{vy}	viable yellow
		A^{1y}	intermediate yellow

	recessive	ob	obese (10)
		ad	adipose (11)
		db	diabetes (12)

polygenic		NZO	New Zealand Obese (13)
		KK	Japanese obese (14)
		C3Hf x I, F_1	
			Wellesley mouse (15)

II. Mice with diabetes and/or obesity caused by external factors.

Diseases induced by artificial means such as :

1. Injury of the hypothalamus

by microsurgery   hypothalamic obese mouse

by chemical means   Gold Thioglucose obese mouse (GTG)

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2. Injury of the islands of Langerhans

    by chemical means   alloxan diabetic mouse

                                 streptozotocin diabetes

3. Disturbance of the hormonal balance

    by administration of corticosteroids

        transplantation of a ACTH secreting tumor

        gonadectomy of the male

        administration of insulin

4. Abnormal feeding or abnormal diets

        high fat diet

        forced feeding

5. Restricting locomotor activity

The mice tabulated under I with the genetically determined dis-
ease are obese as well as diabetic. Among the mice listed under
II, the hypothalamic obese mouse is not diabetic, whereas the
GTG obese mouse is diabetic only under certain conditions. Des-
truction of the islands of Langerhans causes diabetes. An increased
supply of corticosteroids leads in all instances to obesity and
diabetes. Gonadectomy and insulin administration, as well as the
treatments mentioned under 4 and 5 result in the development of
obesity.

Of all genetically determined forms of obesity and diabetes,
the obese-hyperglycemic mouse $(\frac{ob}{ob})$ has been studied most tho-
roughly (for review : 6, 16).

2. A brief historical review of studies on the obese-hyper-glycemic mouse $(\frac{ob}{ob})$.

In 1949, the first obese mouse of this type was found among
the offspring of the V-stock at the Jackson Laboratories, Bar
Harbor, Maine. This new mutation was called obese and desig-
nated by the symbol ob by Ingalls, Dickie and Snell (10).
The most salient features of this syndrome : hyperglycemia,
glucosuria, insulin resistance and hyperphagia were discovered
in 1951 (17, 18). In 1952, hyperplasia and hypertrophy of the
islands of Langerhans was reported (18). Structural changes of
other endocrine organs were not demonstrated at that time. In
the same year, it was reported that obese mice have a very
low oxygen consumption as compared to non-obese mice (20). The
apparent hypometabolism was not obviously reflected in thyroid
activity because iodine uptake appeared to be normal (21). On
the other hand, the animals are very sensitive to cold (22)
and show a low locomotor activity (23), two features which
could result from hypothyroidism. The mice also display hy-
percholesteremia (24).

In the fifties, much attention was paid to the lipid metabolism
of the obese mouse. It was shown that lipogenesis is increased
(25) even under fasting conditions (26) and lipolysis in adi-
pose tissue is reduced (27, 28, 29). The response of adipose
tissue to lipolytic agents such as epinephrine, nor-epinephrine
and ACTH is reduced, as is the response to fasting (28, 29, 30).

In 1963, Mayer reported the presence of glycerol kinase in adi-
pose tissue of obese mice (31) which he considered to be a
fundamental defect.

During the late sixties, there is a rapid succession of crucial publications on the obese mouse. In 1969, Stauffacher and Renold (32) discovered that the diaphragm of their experimental animals was not responsive to insulin, whereas adipose tissue was insulin responsive. On account of these data they suggested that insulin resistance of the muscle tissue is one of the basic abnormalities of the syndrome (33). Two years later, however, Chlouverakis and White pointed out that the adipose tissue of adult obese mice is not responsive to insulin (34). The insulin responsiveness of adipose tissue of obese mice appeared to be age-dependent. This age-dependency in insulin sensitivity was explored in more detail by Genuth et al. (35). They showed that, prior to weaning at 3 weeks of age, diaphragm tissue of obese mice has a normal sensitivity to insulin. Because elevation of the plasma insulin level was the first detectable abnormality in their study, they presented the idea, that a high beta cell sensitivity to carbohydrate stimulation could be a primary event in the genesis of the syndrome.

The investigations of Strautz (36, 37) published in 1968 and 1970, provided strong support for the idea that the primary genetic defect is localized in the islands of Langerhans. His transplantation experiments of islands indicate that in obese islands an essential factor is missing, which is present in normal islands. The absence of this factor should be responsible for the development of hyperinsulinemia, insulin resistance and hyperglycemia.

Looking back at the history of the development of our knowledge about the obese mouse, it is clear that from 1949 until the early sixties, most investigations aimed at a description of the features of the fully developed syndrome in the adult obese mouse. Only after the discovery of the muscle insulin resistance by Stauffacher and Renold in 1969 (32), the obese syndrome was studied in the perspective of development. However, because

the obese mouse can phenotypically, not be distinguished from
its normal littermates before it has reached an age of 4 to
5 weeks, most of the investigations on the development of the
syndrome did not cover younger stages.

3. Time sequence in the occurrence of abnormalities of the development of the syndrome in obese mice.

In the literature 4 phases of the development of the syndrome are distinguished :

1. The asymptomatic phase   – No symptom detectable.
                                   Duration about 3-4 weeks.
2. The dynamic phase of obesity – Rapid weight gain, increa-
                                   sing serum insulin and blood
                                   glucose concentration.
3. The static phase of obesity   – Constant body weight.
4. The phase of the aged syndrome– Weight loss, abnormalities
                                   gradually disappear.

The asymptomatic phase actually ends at that age when the first symptom of the syndrome becomes detectable. It may be clear that a sharp distinction between the subsequent phases does not exist.

Overweight of obese mice was established by Westman (38) to become evident at 26 days and by Chlouverakis et al. at 21 days (39). At 23-25 days, obese mice can be identified with reasonable certainty by assaying the insulin tolerance (38). On account of these data it seems that the asymptomatic phase coincides with the preweaning period which ends at the age of 21 days. After this period, the various symptoms of the syndrome become manifested within a short period of time.

At the age of 4 weeks, an increased insulin tolerance (38), a glucose intolerance as demonstrated by the induction of glucosuria by injections of glucose (40) and hyperinsuline-mia (35, 38) are evident. The age at which hyperglycemia can be demonstrated varies for different laboratories. Whereas Westman (38) reports its demonstration at an age of 4 weeks, Genuth et al. (35) can detect it only after 9 weeks of age. Moreover, whether hyperglycemia will develop depends on the

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genetic background of the mouse strain used (41). Also the insulin resistance of muscle tissue develops during the dynamic phase of obesity, as was shown in 1969 by Chlouverakis and White (34). The age-dependency of this phenomenon was emphasized by these authors and it was studied in more detail by Genuth et al. in 1971 (35), who established that insulin resistance of diaphragm tissue is absent prior to weaning, but well developed at 6 weeks of age.

The third phase of the syndrome is characterized by a constant high body weight. It lasts from about 5 months of age until 13-17 months of age (38).

The fourth phase of the syndrome is characterized by a gradual normalization of the syndrome. The animals lose weight, so that at about 20 months of age, obese and non-obese mice have approximately the same weight. The same holds for plasma insulin and glucose concentration (38), the normalization of which was started already in the third phase.

4. Specific objectives and difficulties of this study.

This investigation should be seen as an attempt to define and localize the primary genetic defect of the obese-hyperglycemic syndrome in the mutant mouse $\frac{ob}{ob}$. It was this aim which placed the accent on the investigation of the syndrome. However, for this type of investigation, the obese mouse is not particularly well suited. The obese mouse is sterile. The only practical way to obtain mice of the genotype $\frac{ob}{ob}$ is to mate mice heterozygous for ob. Furthermore, it is only <u>after 4 weeks</u> that the presence of obesity becomes obvious. Before the age of 4 weeks, the exter- nal phenotype of the obese mouse is not different from the pheno- type of its normal littermates. Since information about the early development was required, experiments had to be carried out with animals of unknown genotype. Animals of the genotype $\frac{ob}{ob}$ could be recognized only afterwards, at about 4 weeks of age. This causes some disadvantages :

1. An extra large number of animals must be studied. The off- spring of the mating $\frac{ob}{+} \times \frac{ob}{+}$, which was used throughout this investigation, is theoretically composed of 25% $\frac{ob}{ob}$ animals (obese phenotype) and 75% $\frac{ob}{+}$ and $\frac{+}{+}$ animals ("normal" littermates). Thus, the control group is three times larger than the experimental group.

2. The animals should not die as a consequence of an experiment.

All animals must stay alive in order to allow the identifica- tion of their genotype at about 4 weeks of age. This simple re- quirement inhibits the performance of certain experiments and ma-. kes other experiments more difficult. Histological and biochemi- cal studies on brain tissue for example, are impossible to carry out with mice younger than 4 weeks because the operation is le- thal. Other operations, such as removal of a piece of pancreas tissue to study structural changes of the islands of Langerhans can be succesfully performed on very young mice but post-operative events, failure of acceptance by the mother or removal of the su- ture may cause the passing away of these nurslings.

In order to approach the goal as outlined above in spite of the limitations mentioned, not only many animals have to be studied, but because the investigation is done with very young mice, microdeterminations, adapted instruments and new techniques were necessary to overcome the problems of the small amounts of material.

The first aim was to determine at what time in the development of the syndrome, the first signs of obesity become manifest. The exact timing of the onset of obesity is an important element for an answer to the question what the causal relation between obesity and hyperinsulinemia is. Is obesity a consequence of, or does it preceed hyperinsulinemia in the obese-hyperglycemic mouse ?

The second aim was to establish whether or not the obese mouse should be considered euthyroid. Although euthyroidism has been suggested by several authors, hypothyroidism can not be excluded on the basis of the data available. After the establishment of hypothyroidism in the adult obese mouse, an attempt was made to determine at what time in development hypothyroidism becomes manifest.

The studies on thyroid function led to the third aim, an investigation of the development of reflexes and behavioral components, of locomotor activity and body growth and a comparison of the time at which these features occur in the obese and non-obese mice.

The fourth aim was to demonstrate that the obese dwarf mouse has the essential characteristics of the obese-hyperglycemic mouse because this would indicate that growth hormone can not play an essential role in the development of the syndrome. A demonstration as such would invalidate an earlier suggestion of Herbai et al. (42, 43, 44).

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Chapter II.

The enlargement of epididymal adipocytes in
relation to hyperinsulinemia in
obese-hyperglycemic mice ($\frac{ob}{ob}$).

Summary

The diameters of epididymal fat cells of 12 to 17 days old
obese and normal littermates were compared following opera-
tive removal of the epididymal fat body. The animals were
kept alive and checked for obesity at an age of 6 weeks.
Fat cells of genetically obese mice began their fast growth
between day 12 and day 14. At this age it is possible to
identify on the basis of fat cell diameters three classes,
representing $\frac{+}{+}$, $\frac{ob}{+}$ and $\frac{ob}{ob}$ respectively, which shows that
the ob allel is incompletely dominant.
Measurements of the plasma insulin concentration revealed
that hyperinsulinemia, which is characteristic for the
obese-hyperglycemic syndrome, is not manifested before the
beginning of the fourth week. Since hyperinsulinemia becomes
evident at a later stage in the development than the rapid
increase in growth rate of the fat cells, it seems that the
insulin resistance and the hyperinsulinemia can not be the
primary cause of the enlargement of the epididymal adipocytes.

Introduction

The monogenic recessive obese-hyperglycemic syndrome is
characterized by the following features : obesity, hyper-
glycemia, insulin resistance and hyperinsulinemia (1).
So far, the age at which obesity begins to develop has not
been accurately established. It is well known that this trait
is externally visible at 3-4 weeks of age. At this stage of
development the fat content of the body is significantly higher
in obese as compared with normal littermates (2). The epididy-
mal fat body is already clearly enlarged at day 16, however
(3).
With respect to the other features it is known that hyperinsu-
linemia is also detectable at an age of 4 weeks (4, 5), where-
as insulin resistance is well established at 6 weeks, but not
detectable at 2.5 weeks and hyperglycemia develops later (5),
although Westman (4) concludes that hyperinsulinemia and hyper-
glycemia develop simultaneously at about 4 weeks of age.
The present study, which is part of a program searching for
the primary effect of the obese gene, aimed at a clear defi-
nition of whether or not obesity does develop before or con-
comitant with the occurrence of hyperinsulinemia.

Material and methods

Experimental animals

The animals used in this study were male obese mice (genotype $\frac{ob}{ob}$) and normal mice ($\frac{ob}{+}$ ard $\frac{+}{+}$) originally derived from the Jackson Memorial Laboratory (Bar Harbor, Maine). The stock was kept in a conditioned laboratory environment and propagated by mating heterozygous parents. The offspring of these matings consists of $\frac{ob}{oo}$, $\frac{ob}{+}$ and $\frac{+}{+}$ animals in the proportions 1 : 2 : 1.

Fat cell diameter measurements

For the study of fat cell growth, cells of the epididymal fat body were used. The fat body was removed under ether anesthesia from animals of 12, 14, 15, 16 and 17 days respectively. The animals were kept alive for the identification of obese and phenotypically normal mice at an age of 6 weeks. Isolated fat cells were obtained by a modification of the method described by Rodbell (6). The fat body was transferred to a 0.15 M NaCl solution, containing 450 units collagenase (Sigma) per ml and incubated for 30 min at 37 ^{o}C with occasional shaking. 50 μl samples of the resulting cell suspension were transferred to a counting chamber with an inner height large enough to enable the cells to retain their original spherical shape.

A microscope equipped with a Leitz camera lucida was used to measure the diameter of 300 randomly chosen fat cells. Because the frequencies of the diameter values were not normally distributed, the median value was used to characterize the diameter of the respective cell populations measured. All computations were made with a Diehl Combitron-S-20 desk computer.

Blood glucose

Blood was obtained from the orbital plexus with a precision capillary (Drummond Sci Co) and deproteinized with a 0.16% uranyl acetate solution in 0.15 M NaCl. The colorimetric determinations are based on a glucose oxidase reaction (Boehringer blood sugar GOD method).

Plasma insulin

Insulin was determined by a radioimmunoassay according to the method C of Hales and Randle (7) with an assay kit available from the Radiochemical Centre (Amersham). Highly purified ox insulin (BDH) was used as a standard. The radioactivity was determined by liquid scintillation counting (Packard Tricarb 3375 and Philips PW 4510-01, efficiency 34%).

Results

Fat cell growth

The primary aim of the experiment was to determine the age at which the epididymal fat cells of genotypically obese mice begin to grow faster than the fat cells of non-obese mice. Since measurements on fat cell diameters of mice of different litters of the same age revealed considerable variations (table 1), probably as a consequence of differences in litter size and milk production of the mothers, comparisons between obese and non-obese ($\frac{oh}{+}$ and $\frac{+}{+}$) fat cells are based upon a calculated percentage of increase in diameter of fat cells in obese relative to those of non-obese (includes homozygous and heterozygous mice) from the same litter. The relation between this index of increase in fat cell diameter and the age of the animals was submitted to a regression analysis. The results are shown in fig. 1, the formula of the regression line being :

$Y = 17.24 \ X - 228.67$; n = 22; r = +0.86; P< 0.001 in which

Table 1.

The relation between litter size and fat cell diameter in
litters at an age of 14 days.

litter nr.	litter size	mean of median values of epididymal fat cell diameters of normal mice in a litter (μ)
18	6	30
19	9	25
20	11	18
21	9	18
22	12	17
23	10	25
24	12	14

Litter size and mean of median values of epididymal fat cell
diameters of normal mice in a litter are negatively correlated.
(d.f. = 5 , r = −0.86 , 0.01 < P < 0.02)

$$Y = \left(\frac{\text{mean of the median (M) of all obese mice in a litter}}{\text{mean of the median (M) of all non−obese mice in a litter}} -1 \right) \times 100$$

X = age in days
n = total number of litters
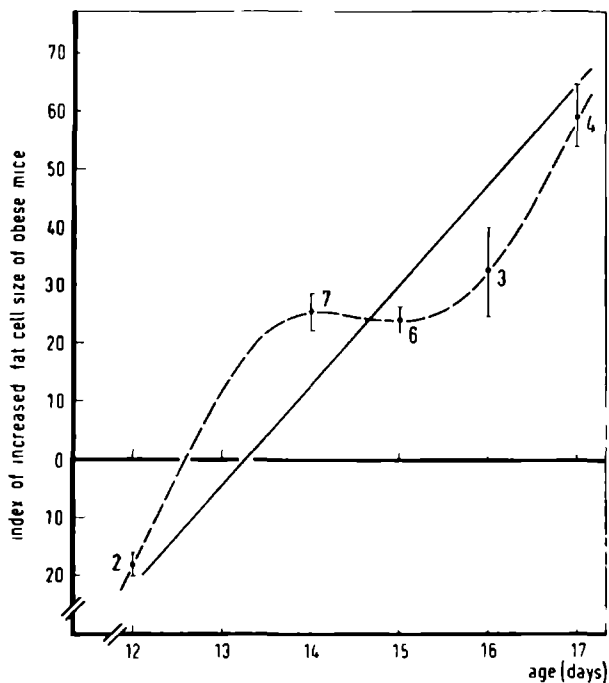r = correlation coefficient
P = probability

Fig. 1

Relative growth of fat cells in the epididymal fat body of
obese mice.

Straight line : calculated regression line.

Curphed graph : Mean of the median values for the index of
increased fat cell size of obese mice. At each point the
SEM is indicated with the number of litters studied.

Although the graph representing the relation between the in-
dex of increase in fat cell diameter in obese and the age de-
viates from the calculated regression line, the correlation
between age and relative fat cell diameter is such $(P<0.001)$
that not too much significance should be attributed to the
deviation which is seen in particular over the period from
day 14 to day 16. In any case, it seems justified to conclude
from these data that the fat cells of homozygous obese mice

begin their enlargement relative to their normal littermates
between 12 and 14 days of age. Therefore, epididymal fat cell
enlargement may be used as a feature to discriminate between
genetically obese and normal littermates at an early stage
of development. In all litters (20) older than 12 days the
mean of the median diameter of obese fat cells was clearly
higher than the mean of M of the normal littermates (paired
analysis, $P < 0.05$).

In some litters inspection of the frequency distribution of
fat cell diameter of littermates at day 14 revealed 3 types :
a small group of animals with small fat cells, a larger group
showing intermediate diameters and another small group of ani-
mals having large cells. Fig. 2 presents an example of a lit-
ter with 3 male mice, one $\frac{+}{+}$, one $\frac{ob}{+}$ and one $\frac{ob}{ob}$ mouse.
At an age of 6 weeks animals of group 3 turned out to be $\frac{ob}{ob}$
animals. The genotype of animals from the other groups was
determined by testmating with heterozygotes, testing the
progeny for obesity. The results are presented in table 2.
The probability to predict $3 \frac{ob}{+}$ and $3 \frac{+}{+}$ by chance is 0.01.
This evidently shows that it is possible to differentiate
between homozygous $\frac{+}{+}$ and heterozygous $\frac{ob}{+}$ on the basis of
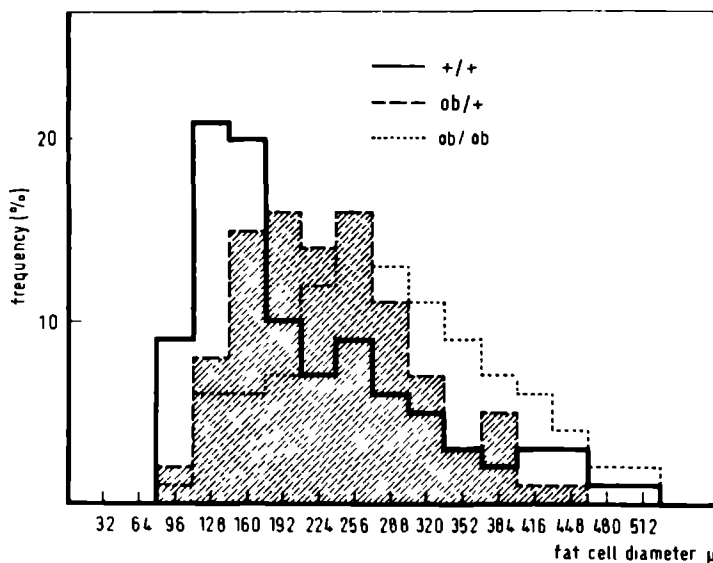diameter values of epididymal fat cells.

Fig. 2. Frequency distribution of fat cell diameters in the epididymal fat body of individual mice. The three genotypes $\frac{+}{+}$ (median value 19 μ), $\frac{ob}{+}$ (median value 24 μ) and $\frac{ob}{ob}$ (median value 29 μ) can be recognized.

Table 2

Results of test-matings of mice with intermediate and low mean diameters of epididymal fat cells with heterozygotes for ob

mouse nr.	fat cell diameter	genotype predicted on the basis of fat cell diameters	obese in the first litter	obese in the second litter
66	interm.	$\frac{ob}{+}$	+	
67	small	$\frac{+}{+}$	−	−
68	interm.	$\frac{ob}{+}$	+	
69	small	$\frac{+}{+}$	−	−
70	small	$\frac{+}{+}$	−	−
71	interm.	$\frac{ob}{+}$	+	

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Blood glucose

Fig. 3, which presents the results of colorimetric determina-
tions of blood glucose levels, indicates that hyperglycemia
becomes evident between 6 and 8 weeks. Only from 8 weeks on
the difference in blood glucose concentration between obese
and normals is significant (Student t-test: t = 3.74, d.f. = 31,
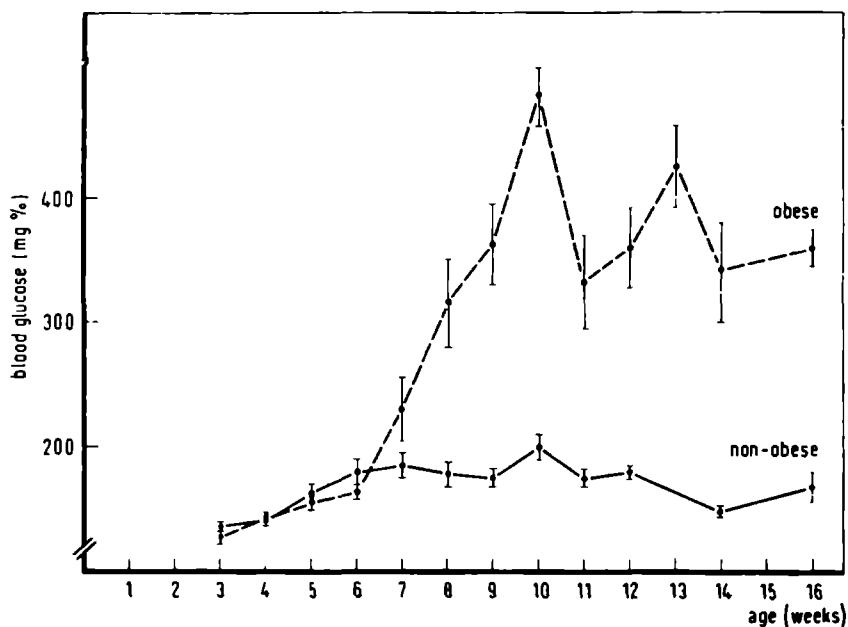P < 0.001).

Fig. 3. Blood glucose concentration of obese and non-obese mice
in relation to age.
From 8 weeks on the difference between obese and non-obese ani-
mals is significant (Student t-test: t = 3.74, d.f. = 31, P < 0.001).
At each point the SEM is indicated.

Plasma insulin

Fig. 4 represents the data of radioimmunoassays of plasma insu-
lin, which reveal that hyperinsulinemia can be detected only
after 4 weeks. The difference between obese and normal mice is
significant from 4 weeks on (Student t-test: $t = 3.60$, d.f. $= 20$,
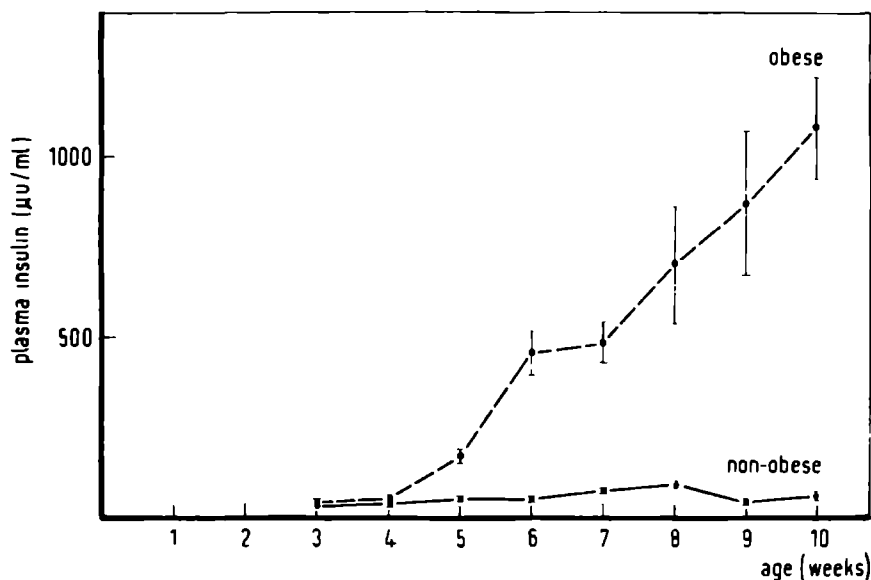$0.001 < P < 0.01$).

Fig. 4. Plasma immunoreactive insulin concentration (expressed in
ox insulin units) in obese and non-obese mice as a function of age.
The difference in insulin concentration between obese and non-obese
animals is significant from 4 weeks on (Student t-test: $t = 3.60$,
d.f. $= 20$, $0.001 < P < 0.01$).
At each point the SEM is indicated.

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Plasma insulin-glucose relationship

In fig. 5 the ratio insulin/glucose is plotted against age.
In obese mice, this index of insulin resistance increases
progressively beginning after 4 weeks, whereas the ratio for
normal mice remains constant at about 0.30. After 6 weeks
the ratio in obese mice is almost 10 times as high as in normal
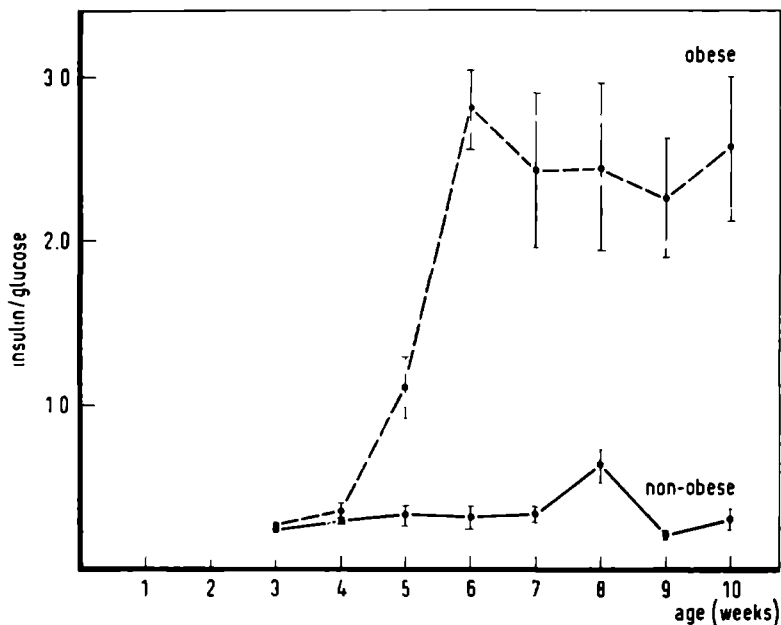littermates.

Fig. 5. The ratio plasma insulin over blood glucose concentra-
tion in obese and non-obese mice in relation to age.
Over the period of development studied, the index of insulin
resistance remains the same for non-obese mice, whereas after
4-5 weeks a clear increase is observed in obese mice.

Obese mice with established obesity have greatly enlarged fat
cells in their epididymal fat body (8). Hyperplasia of epidi-
dymal adipocytes contributes little to the increased weight of
this fat pad (9). Five months old obese mice have cell diame-
ters of around 95μ, normal mice around 71μ (8). (volumes of
44.8 x 10^4 and 18.7 x 10^4 $μ^3$ respectively). At day 14 these
figures are 27μ and 21μ (volumes 1 x 10^4 and 0.5 x 10^4 $μ^3$)
respectively. The difference in fat cell diameter is first
detectable around the 13th day. Since at this early stage of
development heterozygotes can be recognized by their interme-
diate cell size, it seems tnat tne ob allel is incompletely
dominant, in other words that the ob allel is expressed in
the heterozygotes. A similar conclusion may be derived from
the data on the oxidation of glucose in vitro by the epididy-
mal fat body of 3-5 months old mice homozygous or heterozy-
gous for the ob gene (10).

With regard to the question how the relative increase in fat
cell diameters in obese mice is brought about, it could be
suggested that this is a consequence of an increased accumu-
lation of lipids. The increased accumulation could result
either from a higher rate of lipogenesis and/ or a lower
rate of lipolysis in obese fat cells than in normal fat cells.
With respect to lipolysis it is known that under in vitro
conditions lipolysis in the obese fat body is impaired. This
could be due to a lower sensitivity to catecholamines (11, 12).
The in vivo response of obese fat body to catecholamines
appeared to be similar to that of non-obese mice (13). How-
ever, it could be argued that the quantity of free fatty acids,
used as a parameter in these experiments, does not give ade-
quate information about the sensitivity to catecholamines
in mice which differ significantly in the amount of lipids
stored in fat cells. It, thus, cannot be excluded that a re-
duced sensitivity of obese fat cells to catecholamines cau-

ses a reduction in lipolysis with, as a consequence, increase
in intracellular lipid and increased cell growth.
Also high rates of lipogenesis may play a role in the increase
in cell diameter. It is well established that 8-9 week old
obese mice have a strongly increased lipogenesis from acetate
(14). Since insulin is known to influence lipogenesis, it
could be suggested that the increased lipogenesis in obese
mice results from hyperinsulinemia. In the Bar Harbor obese
stock, however, hyperinsulinemia cannot be the cause of the
increased lipogenesis since the hyperinsulinemia becomes ma-
nifest only after 4 weeks (see also: Genuth et al., 1971
(5)), whereas the increase in fat cell diameter begins al-
ready after 13 days. An explanation can be found in the depo-
sition of fatty acids of exogeneous origin in the epididymal
adipocytes of obese mice as shown by Lemonnier et al. (15)
for adult obese mice.
Among the features of the obese-hyperglycemic syndrome, the
increase in fat cell volume appears to be tne first detectable
factor in the development of this syndrome. It remains to
be established, however, which factor(s) are responsible
for the change in lipid metabolism causing the increased
growth rate of the fat cells at an early stage in develop-
ment.

References

1. Bray, G.A. and York, D.A.: Genetically transmitted obesity
    in rodents. Physiol. Rev. 51, 598, 1971.

2. Chlouverakis, C., Dade, E.F. and Batt, R.A.L.: Glucose
    tolerance and the time sequence of adiposity, hyperinsu-
    linemia and hyperglycemia in obese-hyperglycemic mice
    (ob/ob). Meta`olism 19, 687, 1970.

3. Westman, S.: Pathogenic aspects of the obese-hyperglyce-
    mic syndrome in mice (genotype ob/ob). I. Functions of
    the pancreatic β-cells. Diabetologia 6, 279, 1970.

4. Westman, S.: Development of the obese-hyperglycaemic syn-
    drome in mice. Diabetologia 4, 141, 1968.

5. Genuth, S.M., Przybylski, R.J. and Rosenberg, D.M.: In-
    sulin resistance in genetically obese, hyperglycemic mice.
    Endocrinol. 88, 1230, 1971.

6. Rodbell, M.: The metabolism of isolated fat cells. Hand-
    book of Physiology, section 5 Adipose tissue 471, 1965.

7. Hales, N. and Randle, P.J.: Immunoassay with insulin-
    antibody precipitate. Biochem. J. 88, 137, 1963.

8. Hellman, B., Täljedal, I.B. and Westman, S.: Morphologi-
    cal characteristics of the epididymal adipose tissue in
    normal and obese-hyperglycemic mice. Acta Morphologica
    Neerlando-Scandinavica 5, 182, 1962.

9. Johnson, P.R. and Hirsch, J.: Cellularity of adipose de-
    pots in six strains of genetically obese mice. J. Lipid.
    Res. 13, 2, 1972.

10. Yen, T.T.T., Lowry, L. and Steinmetz, J.: Obese locus in
    Mus musculus : A gene dose effect. Biochem. Biophys. Res.
    Comm. 33, 883, 1968.

11. Steinmetz, J., Lowry, L. and Yen, T.T.T.: An analysis of
    the lipolysis in vitro of obese-hyperglycaemic and diabe-
    tic mice. Diabetologia 5, 373, 1969.

12. Mengel, V.K. und Schwabe, U.: Lipolysis an isolierten
    Fettzellen von fettsüchtigen Mäusen (obese mice).
    Arch. Pharmakol. Exptl. Pathol. 260, 176, 1968.

13. Abraham, R.R., Dade,E., Elliott, J. and Hems, D.A.: Hor-
    monal control of intermediary metabolism in obese-hyper-
    glycemic mice. II. Levels of plasma free fatty acid and
    immunoreactive insulin and liver glycogen. Diabetes 20,
    535, 1971.

14. Christophe, J., Jeanrenaud, B., Mayer, J. and Renold, A.E.:
    Metabolism in vitro of adipose tissue in obese-hypergly-
    caemic and goldthioglucose-treated mice. II. Metabolism
    of pyruvate and acetate. J. Biol. Chem. 236, 648, 1961.

15. Lemonnier, D., Winand, J., Furnelle, J. and Christophe, J.:
    Effects of a high-fat diet on obese-hyperglycaemic and
    non-obese Bar Harbor mice. Diabetologia 7, 328, 1971.

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Chapter III.

The role of the thyroid in the development
of the obese—hyperglycemic syndrome in
mice $(\frac{ob}{ob})$.

Summary

Some aspects of thyroid activity in obese mice were investi-
gated. Protein Bound Iodine (PBI), Hormonal Iodine (HI),
Total Thyroxin (TT_4) in plasma, ^{131}I uptake and release
from the thyroid and apical cell width and nuclear volume
of the thyroid epithelial cells were determined in various
age groups in obese and non-obese mice.
On the basis of the results of the comparison between the
obese and non-obese mice it was concluded that the geneti-
cally obese mouse is hypothyroid. This conclusion is suppor-
ted by the finding that body temperature which is low in
obese mice, can be raised by thyroxin injections to normal
values. An increased sensitivity for exogenous thyroxin can
be shown also in the induction of liver mitochondrial α-
glycerophosphate dehydrogenase. The possibility that hypo-
thyroidism is one of the causes of the obese-hyperglycemic
syndrome is discussed.

Introduction

The obese-hyperglycemic syndrome in mice, a recessive
monogenic syndrome discovered by Ingalls et al. in 1950
(1), affects a great variety of metabolic and endocrine
functions (2, 3). A number of the characteristics of this
syndrome, such as high levels of blood cholesterol (4, 5),
increased sensitivity to cold (6, 7), high sensitivity to
thyroxin (8, 9) and low locomotory activity (10), are in-
dicative for a hypothyroidal state. On the other hand, the
basal metabolic rate (BMR) is 44% lower in obese than in
normal mice (8), others reported higher BMR in obese mice
(11, 12). In addition to this discrepancy, histological
studies of the thyroid (13) and the results on ^{131}I uptake
by the thyroid of obese mice failed to provide arguments in
favor of hypothyroidism (14, 15).
Since the thyroid plays an important role in the early post-
natal development of the central nervous system (16, 17)
and interferes with the basal metabolic rate, muscular
activity and lipolysis (18, 19), it could play a significant
role in the development of obesity. It thus appeared essen-
tial to settle the question of whether or not hypothyroidism
does occur in obese mice.

Material and methods

Experimental animals

The animals used in this study were obese (genotype $\frac{ob}{ob}$)
and non-obese littermates ($\frac{ob}{+}$ and $\frac{+}{+}$) originally derived
from the Jackson Memorial Laboratory (Bar Harbor, Maine)
and goldthioglucose obese mice (GTG). In the latter, obesity
was induced by an injection of goldthioglucose at a dose of
1 mg/g at 7 weeks of age.
The stocks were maintained in a conditioned laboratory en-
vironment and propagated by mating heterozygous parents. The
animals were fed an All Hope Farms diet RMH-B containing
0.40 µg iodine per gram. For the experiments with equalized
exogenous iodine supply, mice were fed a iodine-poor diet
with 0.04 µg iodine per gram for 14 days before the experi-
ment was started.

Body temperature

In mice older than 3 weeks rectal temperature was measured
with a TRI-R electronic thermometer model TWL equipped with
probe number 16 (1.5 mm thick) which was inserted 15 mm.
In mice younger than 3 weeks a 1.0 mm thick probe with a
copper-constantane junction was inserted 12 mm. A second
junction was used as a reference and held in a thermostated
waterbath at 37.2^{o}C. The temperature dependent voltage dif-
ference between the two couples was registered by a voltme-
ter in µV, the voltage difference being 18.4 µV per 1° C.

Thyroxin injections

Thyroxin (Fluka) was injected intraperitoneally. The solu-
tion was made 0.15 M NaCl (pH 9.0) containing 2.5 µg thyroxin
per ml. Mice were injected twice a day.

α-Glycerophosphate dehydrogenase assay

Mice were killed by cervical dislocation. The liver was
excised, weighed and homogenized in 0.15 M KCl with a Potter
Elvehjem homogenizer with a Teflon-glass pestle. 0.25 ml
samples from the homogenates (1 g liver/10 ml) were taken
for the manometric assay described by Richert et al. (20).
The O_2-consumption was recorded in $\mu l/min/25$ mg fresh liver
and as $\mu l/min/mg$ protein. Protein determinations were done
after Lowry et al. (1951). Equal samples of homogenate with-
out substrate were used as controls.

Uptake and release of radioiodine from the thyroid

Mice were injected with 5-7 μCi carrier-free $Na^{131}I$ (spec.
act. 6-20 mCi/μg I, Radiochemical Centre, Amersham).
Thyroidal radioiodine content was determined by in vivo count-
ing with a 1.75 inch NaI scintillation detector (Harshaw type
7S8/2EX) coupled to a high voltage supply and activity analyzer
(Philips PW 4620/00 and PW 4633/01). ^{131}I was determined at the
264 KeV gamma emission peak with a window of 50 KeV. Mice were
held by hand with their necks centered above the lead shielded
scintillation detector. The lead shield had a hole of 15 mm
diameter. The efficiency of the equipment was 1.45%.
Corrections were made for background radioactivity, physical
decay of the isotope and non-thyroidal radioactivity.
Thyroidal uptake at 24 hours after injection was expressed as
a percentage of the injected dose. Release studies were started
at 48 hours after injection of the isotope. The thyroidal radio-
iodine content was determined at subsequent days and expressed
as a percentage of the initial count taken at 48 hours after
injection. The relation between time and log of the residual
radioactivity is linear and can be calculated by the method
of least squares. The biological half-life time (t 1/2) was
calculated from the individual regression lines.

Determination of Hormonal Iodine (HI)

The method used was a modification of the method described
by Kreutzer (21) for human plasma. In order to obtain
plasma samples of 0.5-1.0 ml, the blood of 3 mice was
pooled. Samples were run through a column of Dowex-50 Wx2
(200-400 mesh, H^+ form). The column was washed successi-
vely with 8.0 ml N HCl, 8 ml 1.0 M NaCl, 10 ml borate
buffer (pH 8.5) and 4 ml aqua dest. Finally the thyroid
hormones are eluted with 2.5 ml 0.02 N NaOH. In the eluate
of plasma samples of 0.5 ml, 150-300 µg protein is found.
The iodine determination was carried out as follows: to
the eluates and standards $(0-2.10^{-2}$µg thyroxin) 1 ml
5 N H_2SO_4 and 0.2 ml 0.15 N NaClO is added. 10 min later
1 ml arsenious acid is added. The addition of NaClO
caused turbidity which might influence the absorbance
measurements. In order to correct for this effect the
absorbance at 415 mµ was measured before and 30 min after
addition of 0.5 ml ceric reagent. Following exactly 30 min
after the addition of this reagent the absorbance at
415 mµ is read. On semilog paper the relation between con-
centration on the decimal axis and absorbance on the loga-
ritmic axis is linear.

Determination of Protein Bound Iodine (PBI)

PBI was determined by the method described by Barker (22).

Determination of total Thyroxin TT_4

The TT_4 assay is based on the competitive binding method
described by Murphy and Pattee (23). The sensitivity was
increased by using a highly diluted thyroxin-binding
globulin solution prepared from human serum.

Histometry of the thyroid

The excised thyroid was frozen in liquid nitrogen.
After a 4 hour period of temperature equilibration in
the cryostat (Slee, London, type HS) at -18°C, 7 μ
sections were made and stained with Giemsa's solution
(Azur-eosin methyleneblue solution, Merck). Determi-
nations of the follicle diameter, number of epithelial
cells per follicle section and nuclear volume of the
epithelial cells were made after drawing the follicles
and nuclei by means of a microscope equipped with a
camera lucida (Leitz). The arbitrary volume of the
nuclei of the epithelial cells was calculated using
the formula for the prolate spheroid (24):

$$\text{volume} = \text{constant} \times b.l^2. \quad \text{in which:}$$

b = the length of the major axis

l = the length of the minor axis

The apical cell width was calculated with the formula
(25):

$$\text{apical cell width} = \text{constant} \times d.\frac{1}{n} \text{ in which:}$$

d = the inner diameter of the follicle

n = the number of epithelial cells in a given follicle
    section.

Results

Diurnal variation of body temperature

Rectal temperature was recorded during a 24 hour cycle in
9 obese and 9 non-obese male mice, 8 weeks of age (fig. 1).
Body temperature is at its minimum at the transition of
the dark to the light period. Non-obese as well as obese
mice show this diurnal rhythm. The difference in body tem-
perature between the minimum in the light period and the
maximum in the dark period is $2.0^{\circ}C$ in obese and $1.5^{\circ}C$ in
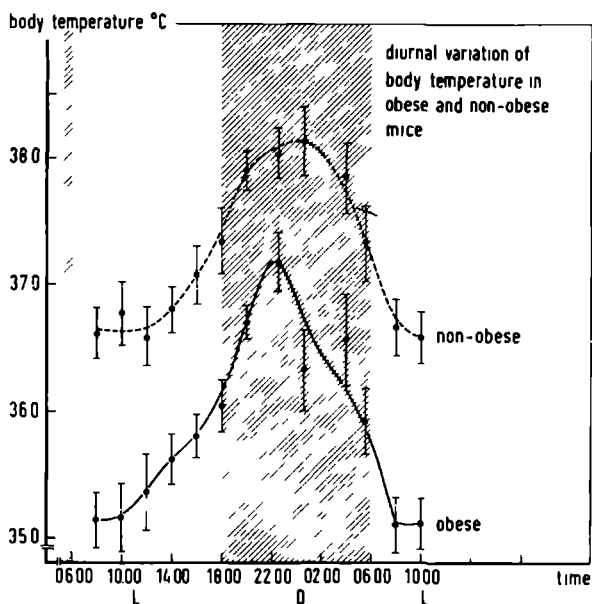non-obese mice.

Fig. 1. Diurnal variation of body temperature of obese and
non-obese mice, 8 weeks of age, during a 24 hour cycle with
fixed light and dark period. The obese and non-obese group
consisted of 9 males each. The mean and the SEM is indicated.

Body temperature in obese and non-obese mice of both sexes

In table 1, body temperature in young and adult obese and non-obese mice are given. It can be seen that the temperature difference between obese and non-obese mice is not sex dependent. Normal female mice have a higher temperature than normal males. This difference in temperature between the sexes is not present between obese females and obese males.

Table 1

Body temperature of obese and non-obese mice of both sexes

age (weeks)	non-obese ♀♀	non-obese ♂♂	obese ♀♀	obese ♂♂
4	37.99 ± 0.09 (12)	37.43 ± 0.23 (14)	36.70 ± 0.06 (10)	36.64 ± 0.07 (11)
8-9	37.80 ± 0.08 (5)	37.42 ± 0.10 (5)	36.43 ± 0.10 (5)	36.46 ± 0.07 (5)

Values represent mean ± SEM. Number of observations in parentheses. Irrespective of the sex, mean body temperature of obese mice is decreased $(P < 0.01)$.
The sex-dependent difference in body temperature between ♀♀ and ♂♂ normal mice $(P < 0.05)$ is not detectable between ♀♀ and ♂♂ obese mice.

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Rectal temperature in mice from the 10th to the 21st day

In 16 litters (50 lean and 15 obese ♂♂) rectal temperature
was determined from day 10 to 21. The results are shown
in fig. 2.

Non-obese control mice show a gradual increase of body
temperature over the period studied. At the age of 3 weeks
they have reached the regular adult body temperature.
Obese mice have a lower temperature than non-obese mice
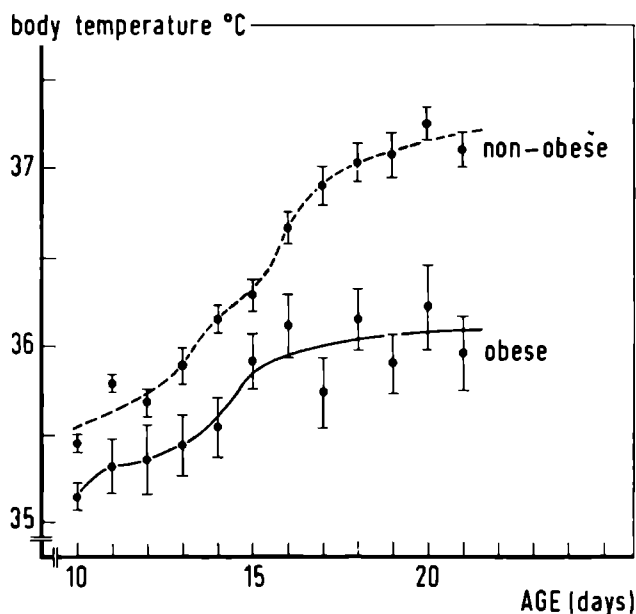from the 10th day on.

Fig. 2. The age dependent body temperature of young obese and
non-obese mice (males). Individual mice were marked at the age
of 10 days and classified in obese and non-obese at 5-6 weeks
of age. The mean and the SEM was calculated from determinations
on 15 obese and 50 non-obese mice. At all ages studied (except
12th and 15th day) obese mice have a lower body temp. compared
with non-obese mice (P < 0.05).

As in the control mice, the body temperature in obese mice increases as they grow older. However, the increase stops at the age of about 16 days. From the 16th day on body temperature remains constant at the adult level. During the period studied, the temperature difference between obese and non-obese mice increases from 0.3 $^{\circ}$C to about 1.0 $^{\circ}$C.

Rectal temperature in mice older than 4 weeks

Rectal temperature was determined in obese and non-obese male mice of 4-20 weeks of age (table 2).

Table 2

Rectal temperature in male mice from 4-20 weeks of age

age (weeks)	obese	non-obese
4	36.64 $\pm$ 0.07 (11)	37.43 $\pm$ 0.09 (12)
5-6	36.09 $\pm$ 0.12 (9)	36.89 $\pm$ 0.18 (10)
8-9	36.46 $\pm$ 0.07 (5)	37.42 $\pm$ 0.10 (5)
9-10	36.34 $\pm$ 0.08 (11)	37.32 $\pm$ 0.10 (12)
20	36.28 $\pm$ 0.10 (11)	37.70 $\pm$ 0.07 (11)
	GTG obese	non-obese controls
20	37.68 $\pm$ 0.09 (10)	37.60 $\pm$ 0.10 (10)

Values are mean $\pm$ SEM. Number of observations in parentheses. At all ages, the difference in temperature between obese and non-obese mice is significant (P< 0.01), except in GTG obese mice.

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It can be seen that obese mice have a significantly lower body temperature than non-obese mice, the difference being on the average 1.0 $^{\circ}$C. GTG obese mice, on the contrary, do not have a lower body temperature as compared to the controls.

Histometrical observations of the thyroid

Histometrical determinations of apical cell width and nuclear volume in the age groups 5-6 and 9-11 weeks indicate hyperactivity of follicle epithelial cells in obese mice as compared to the controls. For example at 5-6 weeks, log nuclear volume is 1.93 ± 0.02 in obese and 1.86 ± 0.01 in lean mice. Apical cell width values are 5.64 ± 0.04 and 6.00 ± 0.03 respectively (arbitrary units). No differences could be detected in younger mice.

Concentration of HI, PBI and TT_4 in plasma

The values of these parameters are given in table 3. At all ages studied, the concentration of HI in obese mice is significantly lower than in non-obese mice. This difference amounts to 30-40%. With respect to PBI and TT_4 in obese mice of 5 months, a similar decrease has been detected.

Table 3

Plasma concentrations of HI, PBI and TT_4 and ^{131}I uptake and release of the
thyroid in obese and non-obese mice at various ages

age (weeks)	animals	HI ($\mu g/100$ ml)	PBI ($\mu g/100$ ml)	TT_4 ($\mu g/100$ ml)	^{131}I uptake	t1/2 thyroidal ^{131}I
4	obese				9.86 ± 1.47 (9)**	3.33 ± 0.34 (9)**
	non-obese				18.79 ± 1.80 (9)	2.09 ± 0.31 (9)
5- 6	obese	0.55 ± 0.10 (9)**				
	non-obese	0.92 ± 0.10 (6)				
9-11	obese	0.39 ± 0.10 (7)**		1.43 ± 0.45 (4)	7.40 ± 0.37 (11)**	2.79 ± 0.21 (11)**
	non-obese	0.66 ± 0.07 (11)		1.83 ± 0.32 (4)	15.34 ± 2.08 (11)	4.13 ± 0.35 (11)
20-24	obese	0.98 ± 0.06 (5)	1.60 ± 0.11 (7)**	1.28 ± 0.14 (4)**	6.81 ± 0.64 (15)**	3.96 ± 0.47 (10)
	non-obese	1.37 ± 0.11 (5)**	2.31 ± 0.17 (6)	1.86 ± 0.10 (5)	15.27 ± 0.74 (15)	3.95 ± 0.55 (10)

Values are mean $\pm$ SEM. Number of observations in parentheses.

HI and PBI are expressed as μg $I/100$ ml plasma and TT_4 as μg thyroxin/1oo ml plasma.

^{131}I uptake is expressed as the percentage of the injected dose at 24 hours after the injection.

As a parameter for turn over of thyroidal radioiodine, the biological half-life is

indicated and expressed in days.

** differs from non-obese value at $P < 0.01$.

Uptake and release of ^{131}I by the thyroid in mice fed a diet
with normal iodine content

The uptake of ^{131}I in obese mice is lower than in non-obese
mice at all ages studied (table 3, fig. 3). The thyroid of
obese mice incorporates only half the quantity of radioiodi-
ne incorporated by the non-obese thyroid. The release of
radioiodine from the thyroid of obese mice is slower at 4
weeks, faster at 9-11 weeks and occurs at the same rate at
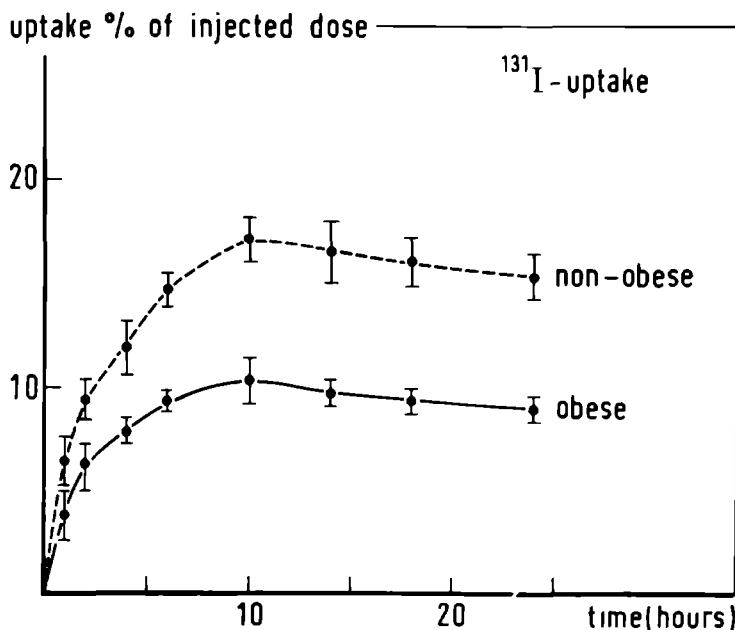20-24 weeks compared to the non-obese (table 3, fig. 4, 5
and 6).

Fig. 3.   [131]I uptake by the thyroid in obese and non-obese
mice, 5 months of age, on a normal iodine-rich diet. Uptake
is expressed as the percentage of the injected dose. The
mean and the SEM were calculated from determinations cn
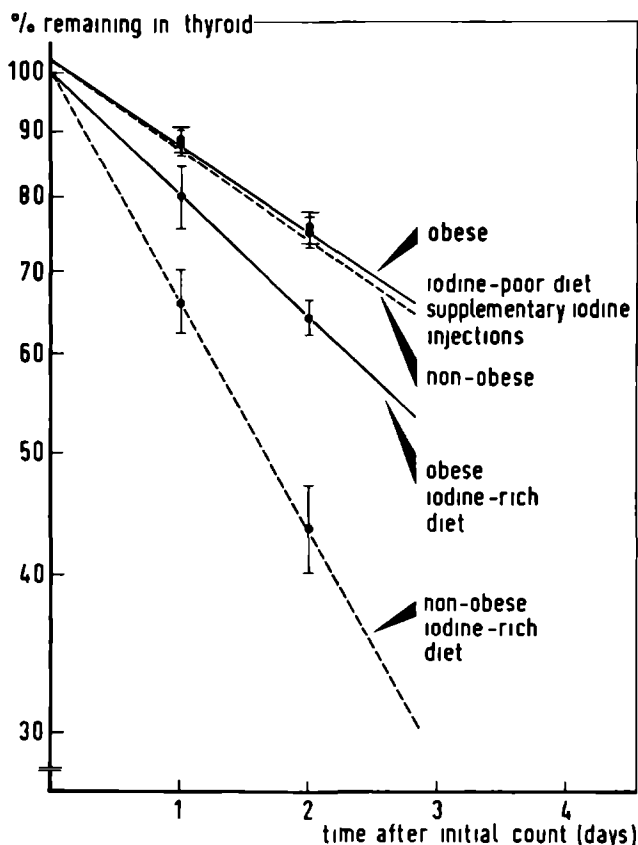5 obese and 5 non-obese male mice.

Fig. 4. ^{131}I release from the thyroid in obese and non-obese mice, 4-5 weeks of age, under two sets of conditions. In one experiment the animals were fed a normal iodine-rich diet (9 obese and 9 non-obese male mice) and in the second experiment another group of mice was fed a iodine-poor diet (15 obese and 14 non-obese male mice) and supplemented with a daily injection of 2 μg iodine. Determinations of thyroidal radioiodine were started at 48 hours after injection of 5-7 μCi of the radioactive isotope. At the subsequent days radioiodine content was determined and expressed as a percentage of the 48 hour count. The mean and the SEM is indicated.
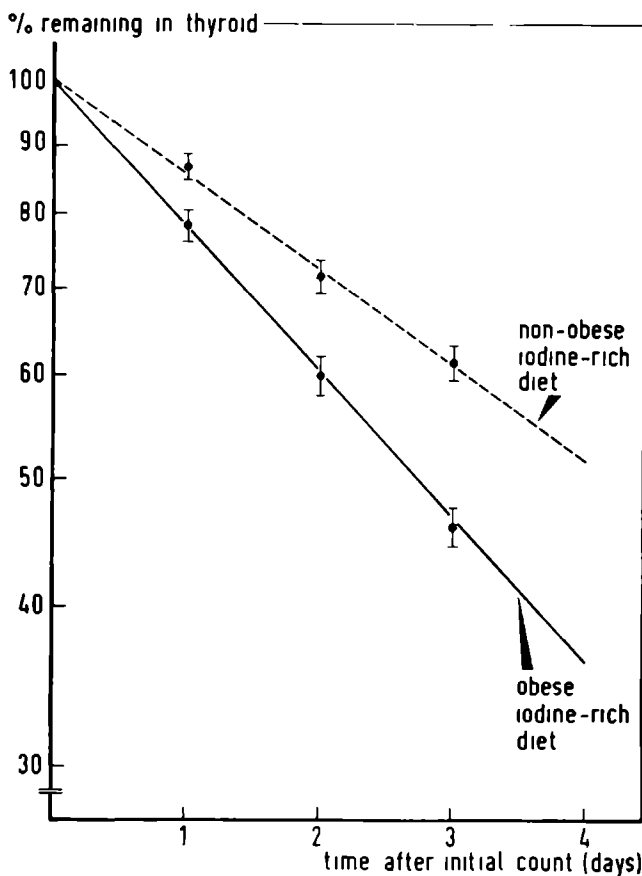
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Fig. 5. [131]I release from the thyroid of obese and non-obese mice, 9-11 weeks of age, fed a normal iodine-rich diet. The experimental group consisted of 11 obese and 11 non-obese male mice. The mean and the SEM is indicated.
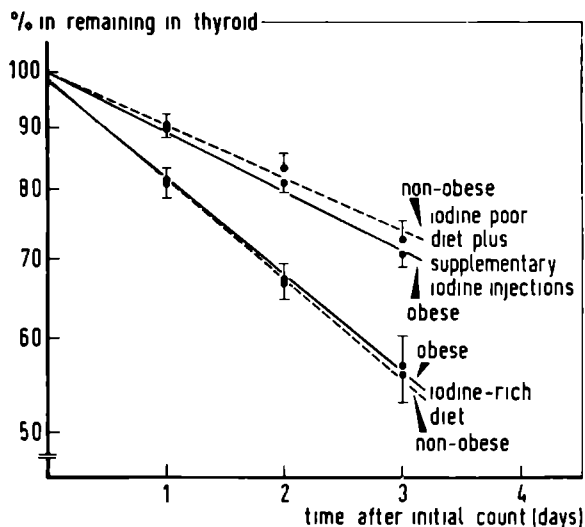
Fig. 6. [131]I release from the thyroid of obese and non-obese mice, 20-24 weeks of age, under two sets of conditions : the first group (10 obese and 10 non-obese males) was fed a normal iodine-rich diet and tne second group (9 obese and 9 non-obese males) was fed a iodine-poor diet and supplemented with a daily injection of 2 μg iodine.

Uptake and release of ^{131}I by the thyroid in mice fed a diet with low iodine content and supplemented with iodine injections of 2 μg per day

Under standerdized dietary conditions (low iodine), obese and non-obese mice were supplied with 2 μg of iodine per day. Under these conditions thyroidal uptake of radioiodine is significantly lower in obese mice than in non-obese mice. At 4 weeks the iodine uptake is 23% lower and at 20-24 weeks 49% lower than in non-obese mice of the same age (table 4). The biological half-life of thyroidal radioiodine does not differ between obese and non-obese mice under these conditions. The rate of release is lower in both types of mice as compared to the animals fed a normal diet (table 4, fig. 4, 5 and 6).

Table 4

^{131}I uptake and release of the thyroid in obese and non-
obese mice fed an iodine deficient diet and injected with
2 µg iodine per day.

age (weeks)	animals	^{131}I uptake	^{131}I release (t 1/2)
4-5	obese	5.18 ± 0.22 (15)**	4.76 ± 0.28 (15)
	non-obese	6.70 ± 0.54 (14)	4.73 ± 0.36 (14)
20-24	obese	4.60 ± 0.21 (9)**	6.18 ± 0.26 (9)
	non-obese	9.10 ± 0.85 (9)	7.51 ± 0.76 (9)

Values are mean $\pm$ SEM. Number of observations in parentheses.
^{131}I uptake is expressed as the percentage of the injected
dose 24 hours after injection. ^{131}I release (t 1/2) is ex-
pressed as the biological half-life of thyroidal radioiodine
in days. The animals were pretreated by feeding iodine-poor
diet and injecting 2 µg I per day during 14 days before the
experiment was started.

** differs from non-obese value at P< 0.01.

Effect of thyroxin injections on body temperature
The sensitivity of o ese and non-obese mice to thyroxin with
respect to body temperature was studied in 2-3 months old
mice. Non-obese mice were injected with 6 µg thyroxin/100 g
/day in a split dose. One obese group received the same dose
and another group 3 µg/100 g/day. The absolute amount of
thyroxin injected in obese mice as compared to the non-obese
mice is the same in the 3 µg/100 g/day group, but twice as
high in the 6 µg/100 g/day group. The response of the body
temperature in obese mice did not differ between the two

groups. Body temperature began to rise after 4 days (fig. 7).
After about 2 weeks the response was at its maximum. The
increase in obese mice is 1.6 $^{\circ}$C and in non-obese mice
0.8 $^{\circ}$C. Under these conditions obese mice can attain the
body temperature of non-injected non-obese mice.

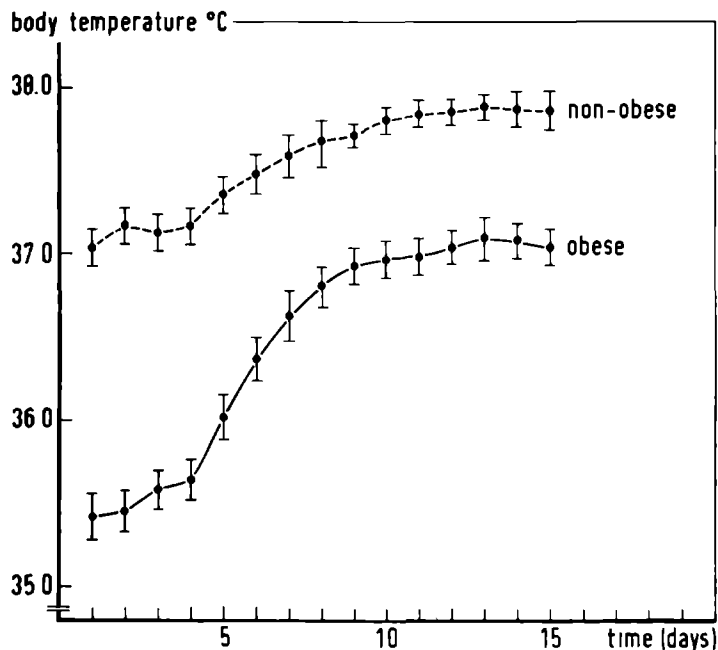Fig. 7. The influence of thyroxin injections on body tempe-
rature in obese and non-obese mice, 2-3 months of age.
18 non-obese mice were injected 6 µg thyroxin/100 g/day.
9 obese mice received an identical dose and another group
of 9 obese mice was injected 3   thyroxin/100 g/day.
The response of the two obese groups was similar. There-
fore, the observations were pooled.
The mean and the SEM is indicated.

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Induction of α-glycerophosphate dehydrogenase by thyroxin

10 obese and 5 non-obese mice, 12 weeks old, received thyroxin injections every day in a split dose. The dosage was the same as in the previous experiment. After 4 days the GPDH activity was determined. The increase in enzyme activity in obese mice was significantly larger than in non-obese mice, irrespective of the dose of thyroxin applied (6 or 3 µg/100 g/day) and the reference used for quantification of enzyme activity (per 25 mg liver or per mg protein) (table 5). In each experimental group body temperature rose significantly during these 4 days.

Table 5

Induction of GPDH in obese and non-obese mice.

animals	thyroxin dose	GPDH act. before injection		after 4 days injections		increase	
		per 25 mg liver	per mg protein	per 25 mg liver	per mg protein	per 25 mg liver	per mg protein
non-obese	6	1.61 ± 0.13	0.48 ± 0.05	2.19 ± 0.08	0.58 ± 0.03	36%	21%
obese	3	1.84 ± 0.10	0.26 ± 0.03	3.42 ± 0.13	0.42 ± 0.04	86%	71%
obese	6			3.16 ± 0.11	0.44 ± 0.03	72%	62%

GPDH activity is expressed as $\mu L\ O_2/min/25$ mg fresh liver or per mg protein.

The increase in activity after 4 days is significant at the 1% level (Student t-test).

The mean and the SEM are indicated.

All values are averages of 5 measurements in different animals.

Discussion

Soon after the discovery of the obese-hyperglycemic syndrome
in mice (1), attention was focussed on the thyroid because of
the fact that this endocrine organ with its well known influ-
ence on the basal metabolic rate (BMR) could be an important
factor in the development of this complicated syndrome.
Mayer et al. (8) discovered that the BMR of obese mice is
44% lower as compared with the non-obese controls. Although
it is reasonable to assume that this is a consequence of a
hypoactive thyroid, no aberrations of the histological pic-
ture (13), nor a lower ^{131}I uptake of the thyroid could be
detected (14, 15). Since arguments in favor of, but also in
contrast to, an influence of the thyroid on the syndrome can
be found in the literature (8, 11, 12, 13, 14, 15), it seemed
worthwile to clarify this question.
The present results on the concentration of HI, PBI and TT_4
(table 3) in plasma of obese and non-obese mice clearly show
that the concentration of thyroxin in the bloodplasma is
30-40% lower in obese mice. These results indicate that the
obese mouse is hypothyroid, at least from 5 weeks on.
In mice with hypothyroidism, one would expect a lower uptake
and a slower release of radioiodine from the thyroid than in
euthyroid mice. In fact, a decreased uptake in obese mice was
found at all ages studied (table 3, fig.3). The uptake of
radioiodine by the thyroid in obese mice was on the average
only 50% of the uptake measured in non-obese mice.
The results on radioiodine release are less clear. The expec-
ted low rate of release was not found in all age groups. The
interpretation of the results presented in fig. 4, 5 and 6
on the release of radioiodine is complicated by differences
in dietary intake of ^{127}I which may cause differences in pool-
size of ^{127}I and therefore differences in specific activity
of ^{131}I. Hyperphagia of obese mice is very well known (9, 10,
26). In the stock used, young obese mice show a hyperphagia

of 40%, and old obese mice eat about 20% more than the non-obese controls. As a consequence of the hyperphagia the endogeneous specific activity of ^{131}I in obese mice will be lower than in normal mice. This in itself will lead to a lower uptake of radioiodine by the thyroid of obese as compared with non-obese when the state of thyroid activity is comparable. In order to eliminate this influence, the animals were fed a iodine deficient diet and supplemented with iodine by a daily injection of 2 μg iodine (2.62 μg KI). This results in an equal supply of iodine for obese and non-obese mice. Following this treatment the thyroidal uptake of ^{131}I by obese mice was decreased as compared to that of non-obese which agrees with the suggestion that obese mice are hypothyroid. The influence of the dietary iodine is also demonstrated by a comparison of the uptake values presented in table 3 and 4 which shows that part of the difference in ^{131}I uptake between obese and non-obese mice receiving a normal diet is caused by the difference in ^{127}I intake.

As shown in figs. 4, 5 and 6, as in table 4 obese and non-obese mice with an experimentally equalized iodine supply displayed a similar rate of radioiodine release from the thyroid. As such this result seems to be in contrast with the other data indicating hypothyroidism in obese mice. However, since the release of radioiodine was measured in obese as well as non-obese mice relative to the radioiodine content at 48 hrs after injection, the relative rate of iodine release was established. These data do not permit conclusions as to the absolute release of iodine by obese as compared to non-obese thyroids. In other words, the data showing the relative rates of radioiodine release do not necessarily disagree with hypothyroidism in obese mice.

It should be pointed out that the results of histometrical analysis of the thyroid follicles indicate hyperactivity rather than hypoactivity of the epithelial cells. It cannot be excluded, however, that as a consequence of fatty infiltration

into the thyroid the number of follicles in obese is lower
than in normal mice resulting in a hyperactivity of the indi-
vidual epithelial cells.

Hypothyroidism should result in a lower BMR. This has indeed
been observed (8). The lower body temperature of obese mice
(table 2), reflects a lower BMR. GTG obese mice, which are
euthyroid (27) have a normal temperature (table 2). In obese
mice the temperature difference is not dependent of sex (table
1). It was observed in male as well as in female obese mice.
The diurnal variation of temperature in obese mice follows
the pattern seen in control mice with a maximum during the dark
period and a minimum during the light period (fig.1). The me-
tabolic state of the animals during the light period can be
considered as near basal because the animals show very low
feeding and locomotor activity. It is interesting to notice
that during this period, the temperature difference between
obese and non-obese mice is at its maximum of 1.4 $^{\circ}$C.
Hypothyroidism is characterized by an increased sensitivity
for thyroid hormones. We tested the sensitivity of obese and
non-obese mice for exogeneous thyroxin with respect to body
temperature and the induction of liver mitochondrial α-glyce-
rophosphate dehydrogenase (fig. 7 and table 5). The expected
hypersensitivity was confirmed : body temperature rose 1.6 $^{\circ}$C
in obese and 0.8 $^{\circ}$C in non-obese mice during a treatment with
thyroxin, whereas the relative increase of liver GPDH activity
was twice as high in obese as compared to non-obese mice.
These results again support the idea that the obese mouse is
hypothyroid. So far, conclusive evidence about the thyroidal
status is limited to mice older than 4 weeks. Information
about mice younger than 4 weeks is only indirect. The body
temperature in obese mice is decreased from the 10th day on
(fig. 2). The increase in body temperature during the period
studied reflects the development of homeothermia. The low bo-
dy temperature of these young obese mice could reflect a low
BMR as compared to the non-obese mice. The results on O_2 con-

sumption of obese mice of 14–28 days as reported by Fried et al.
(28) can be interpreted similarly. Both parameters, body tempera-
ture and O_2 consumption could be consequences of a decreased BMR
resulting from hypothroidism.

Hypothyroidism may be the explanation for various aspects of the
obese-hyperglycemic syndrome. It has a calory saving effect resul-
ting from a decreased BMR and a decreased locomotor activity.
Together with the hyperphagia, this results in a very positive
caloric balance which might explain the overweight. The decrea-
sed sensitivity of the lipolytic process to fasting, epinephrine
and other lipolytic factors in obese mice may be another result
of hypothyroidism (18, 19). Other aspects of the syndrome that
can be explained by a hypofunction of the thyroid are the high
concentration of cholesterol (4, 5), the high sensitivity to
cold (6, 7) and abnormalities in the skin (13). Recently, Kozak
(29) reported a decreased brainweight in obese mice, which pro-
bably results from a decreased number of cells. Since the thyroid
hormone is considered to be a hormone affecting postnatal deve-
lopment of the central nervous system, the defective development
of the brain in obese mice can very well be a result of conge-
nital hypothyroidism.

Acknowledgements

References

1. Ingalls, A.M., Dickie, M.M. and Snell, G.D.: Obese, a new mutation in the house mouse. J. Heredity 41, 317, 1950.

2. Bray, G.A., and York, D.A.: Genetically transmitted obesity in rodents. Physiol. Rev. 51, 598, 1971.

3. Stauffacher, W., Orci, L., Cameron, D.P., Burr, I.M., and Renold, A.E.: Spontaneous hyperglycemia and/or obesity in laboratory rodents: an example of the possible usefulness of animal disease models with both genetic and environmental components. Rec. Progr. Horm. Res. 27, 41, 1971.

4. Mayer, J. and Jones, A.K.: Hypercholesteremia in the hereditary obese-hyperglycemic syndrome of mice. Amer. J. Physiol. 175, 339, 1953.

5. Zomzely, C. and Mayer, J.: Levels of serum cholesterol in obese mice. Nature 182, 1738, 1958.

6. Mayer, J. and Barrnett, R.J.: Sensitivity to cold in the hereditary obese-hyperglycemic syndrome of mice. Yale J. Biol. Med. 26, 38, 1953.

7. Davis, T.R.A. and Mayer, J.: Imperfect homeothermia in the hereditary obese-hyperglycemic syndrome of mice. Amer. J. Phsiol. 177, 222, 1954.

8. Mayer, J., Russell, R.E., Bates, M.W. and Dickie, M.M.: Basal oxygen consumption of hereditarily obese and diabetic mice. Endocrinol. 50, 318, 1952.

9. Mayer, J., Russell, R.E., Bates, M.W. and Dickie, M.M.: Metabolic, nutritional and endocrine studies of the hereditarily obesity-diabetes syndrome of mice and mechanism of its development. Metabolism 2, 9, 1953.

10. Mayer, J., Marshall, N.B., Vitale, J.J., Christensen, J.H., Mashaekhi, M.B. and Stare, F.J.: Exercise, food intake and body weight in normal rats and genetically obese adult mice. Amer. J. Physiol. 177, 544, 1954.

11. McClintock, R. and Lifson, N.: CO_2 output and energy balance of hereditary obese mice. Amer. J. Physiol. 189, 463, 1957.

12. McClintock, R. and Lifson, N.: Measurement of basal and total metabolism in hereditarily obese-hyperglycemic mice. Amer. J. Physiol. _193_, 495, 1958.

13. Bleisch, V.R., Mayer, J. and Dickie, M.M.: Familial diabetes mellitus in mice, associated with insulin resistance, obesity and hyperplasia of the islands of Langerhans. Am. J. Pathol. _28_, 369, 1952.

14. Wykes, A.A., Christian, J.E. and Andrews, F.N.: Radioiodine concentration and thyroid weight in normal, obese and dwarf strains of mice. Endocrinol. _62_, 535, 1958.

15. Goldberg, R.C. and Mayer, J.: Normal iodine uptake and anoxia resistance accompanying apparent hypometabolism in hereditary obese-hyperglycemic syndrome. Proc. Soc. Exptl. Biol. Med. _81_, 323, 1952.

16. Shapiro, S.: Metabolic and maturational effects of thyroxine on the infant rat. Endocrinol. _78_, 527, 1966.

17. Eayrs, J.T.: Influence of the thyroid on the central nervous system. Britt. Med. Bull. _16_, 122, 1960.

18. Debons, A.F. and Schwartz, I.L.: Dependence of the lipolytic action of epinephrine in vitro upon thyroid hormone. J. Lipid Res. _2_, 86, 1961.

19. Goodman, H.M. and Bray, G.A.:Role of the thyroid hormones in lipolysis. Amer. J. Physiol. _210_, 1053, 1966.

20. Richert, D.A., Schenkman, J. and Westerfeld, W.W.: An anti-thyrotoxic assay based upon the response of rat liver α-glycerophosphate dehydrogenase. J. Nutr. _83_, 332, 1964.

21. Kreutzer, E.K.J.: Determination of hormonal iodine. Clin. Chim. Acta _37_, 519, 1972.

22. Barker, S.B.: Determination of protein bound iodine. J. Biol. Chem. _173_, 715, 1948.

23. Murphy, B.E.P. and Pattee, C.J.: Determination of thyroxine utilizing the property of protein binding. J. Clin. Endocr. Metab. _24_, 187, 1964.

24. Palkovits, M. and Fischer, J.: Karyometric investigations. Akadémiai Kiadó. Budapest, 1968.

25. Lever, J.: Onderzoekingen betreffende de schilklierstructuur. Dissretation. The Hague, 1950.

26. Fuller, J.L.: Genetic aspects of regulation of food intake. Adv. Psychosom. Med. <u>7</u>, 2, 1972.

27. Schindler, W.J. and Liebelt, R.A.: Thyroid activity in mice with hypothalamic lesions induced by goldthioglucose. Endocrinol. <u>80</u>, 387, 1967.

28. Fried, G.H. and Antopol, W.: Oxygen consumption in litters of obese hyperglycemic mice. Fed. Proc. <u>22</u>, 668, 1963.

29. Kozak, L.P.: Neurochemical correlates of obesity. 43rd annual report 1971-1972 of the Jackson Laboratory, p. 83.

Chapter IV.

Growth pattern and behavioral traits associated
with the development of the obese-hyperglycemic
syndrome in mice ($\frac{ob}{ob}$).

Summary

With respect to the development of various reflex responses
and the time of eye opening, the postnatal development of
obese mice is normal. From the beginning of the second week
low locomotory activity is detectable in genetically obese
mice. This is one week before the epididymal adipocytes of
genetically obese mice start to enlarge compared to non-
obese mice. From the first day of postnatal development, the
growth rate of obese mice is increased as compared to the
non-obese littermates.
Indications were obtained which favor the idea that mutant
nurslings are congenitally hyperphagic. The caloric balance
of obese mice, from birth till weaning, and its composing
elements is discussed.

The mutant mouse obese (ob/ob) was isolated in 1949 at the
Jackson Laboratory. The obese-hyperglycemic syndrome is
inherited as a single recessive factor (1). It is character-
ized by many aberrations among which: obesity, hyperglycemia,
insulin resistance, hyperphagia, decreased locomotory activity
and infertility (2, 3). Recently, a decreased concentration
of thyroxin in blood plasma of obese mice was found (4). In
combination with other evidence, this finding led to the con-
clusion that obese mice are hypothyroid.
Hypothyroidism could account for a number of characteristics
of the syndrome: low basal metabolic rate, low body tempera-
ture, high sensitivity to cold, high concentration of blood
cholesterol and high sensitivity to exogeneous thyroxin.
In addition to the low basal metabolic rate resulting from
hypothyroidism, increased food intake and low locomotory acti-
vity may give rise to the extremely positive caloric balance
of young obese mice. The sequence in which these features
become manifest during the development of the syndrome is not
yet clear. In particular the causal relationship between low
locomotory activity and the development of obesity is subject
of discussion (5, 6). In order to obtain some insight in this
relationship, a study of locomotory activity and body growth
during the early period of postnatal development was made.
Because thyroid hormones are known to interfere with the devel-
opment of the central nervous system (7, 8) and congenital
hypothyroidism of obese mice was indicated (4), it seemed
worthwhile to examine if the time of occurrence of certain re-
flexes and other features during the development of the obese
mouse differs from that of normal mice.

Material and Methods

Experimental animals

The experimental animals descent from an obese strain ob-
tained from the Jackson Laboratory in 1958. Because ob/ob
mice are sterile under normal conditions, matings between
heterozygous parents have to be made.
The ob/ob mice can be distinguished from their normal
littermates at the age of 4-5 weeks. Since we started our
experiments with 1 day old animals, the offspring was mark-
ed individually and checked for obesity at 4-5 weeks.

Locomotory activity and reflexes

Because the activity of young mice is significantly in-
fluenced by the environmental temperature all studies on
locomotory activity were carried out in a plastic test cage
in which the temperature was maintained at 27-29°C by means
of a red heating lamp. Individual mice were placed in the
cage, the bottom surface of which was divided in squares of
4 x 4 cm, for 5 min. From the 17th day on 8 x 8 cm squares
were used to allow for a high activity of the mice. Activity
was expressed as the number of line crossings per 5 min. A
crossing was scored when 3 limbs were placed in an adjacent
square.
The development of reflex responses; postural flexion, rooting,
cliff drop aversion, righting, negative geotaxis, bar holding,
vibrissae placing, fore-limb placing, hind-limb placing, fore-
limb grasping, hind-limb grasping, pain reaction and postural
extension (see Fox 1965)(9), behavioral components; pivoting
and straight line walking (Van Abeelen and Kalkhoven, 1970)
(10), and the time of eye-opening were established by obser-
vations on day 2, 3, 4, 5, 7, 9, 10, 12, 13, 14, 15, 16, 17,
18 and 20 in 60 mice.

Intake of solid food

Six obese and 6 non-obese mice of 5 weeks of age and 14
obese and 16 non-obese mice of 5 months of age were
caged individually. A fixed quantity of food pellets was
put in the food hopper and weighed every 24 hours. The
average food intake and the efficiency of the conversion
into body weight was calculated from the individual ave-
rages over 3 days.

Body weight

Each of a group of 100 mice was weighed every day from
birth untill the 16th day. The data obtained from 9 ani-
mals were not taken into account because they died during
the test period. The remaining 91 mice included 65 non-
obese and 26 obese mice. The mean body weight as well as
the relative growth rate which is defined as the percen-
tage of increase of body weight as compared to the weight
of the previous day was calculated for each day.

Results

Locomotory activity

The method used for establishing locomotory activity can
be adequately applied to animals which are at least 7 days
old. During the second week after birth, locomotory activity
of obese mice is lower than that of non-obese mice (fig. 1,
Wilcoxon's Two Sample Test $p < 0.05$) (11).
During the third week of life, locomotory activity of obese
mice is not significantly different from that of non-obese
littermates. However, from day 21 the locomotory activity in
obese mice is lower again.
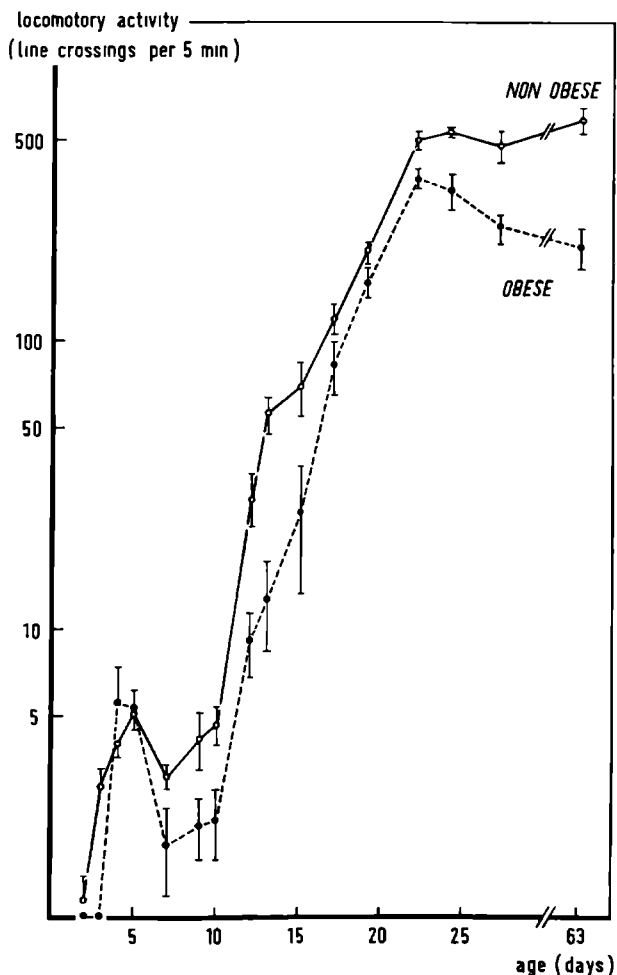
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Fig. 1. For this study 60 mice were used, 51 of which were non-obese and 9 were obese mice. Because no significant differences in the activity of male and female mice could be established, results of the two sexes were pooled. Locomotory activity was expressed as the number of line crossings per 5 min with squares of 4 x 4 cm. The mean and the SEM is indicated logorithmically.

Reflexes, behavioral components and eye-opening

Studies on the development of the reflexes postural extension,
rooting, fore-limb placing, hind-limb placing, fore-limb
grasping, hind-limb grasping, bar holding, cliff drop aversion,
pain reflex and vibrissae placing revealed no obvious differ-
ences between obese and non-obese mice. The retardation in the
development of righting, pivoting and straight line walking is
slight, but insignificant (fig. 2). Also, the process of eye-
opening proceeds equally between obese and normal mice.

Food intake and efficiency of weight gain

Obese mice in the dynamic phase of obesity have a 44% higher
food intake than non-obese mice. In the static phase of obesity,
obese mice are only moderately hyperphagic (table 1). Obese
mice of 5 weeks of age transform 19% of the food consumed into
body weight. The efficiency of non-obese mice of this age is
only 13%.

Absolute changes of body weight

The difference between the body weight of obese and non-obese
mice increases from the day of birth on. It reaches significance
at day 10 (paired analysis, $p < 0.05$).

Relative increase of body weight

The relative weight gain was calculated per day for obese and
non-obese mice. Observations on male and female mice were pooled
because they did not differ during the period studied (fig. 3).
In obese mice the relative growth rate is almost permanently
higher than in non-obese mice.
The growth rate of obese mice becomes significantly higher at day
6-7 ($p < 0.05$). This significancy is temporarily lost at day
10-11 till day 13-14, but is reestablished afterwards.
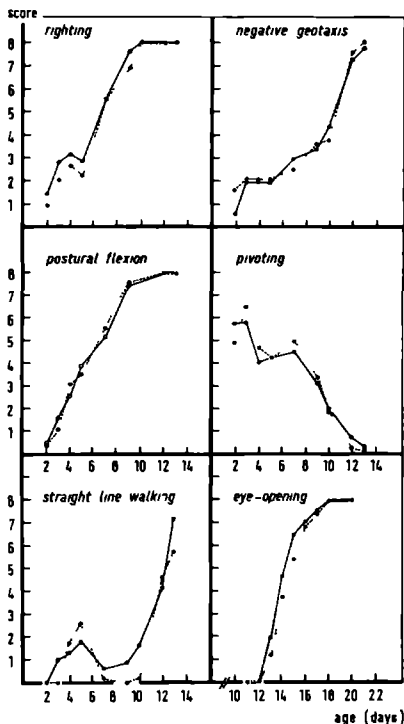
Fig. 2. For the study of righting, negative geotaxis, postural flexion, pivoting and straight line walking 51 non-obese and 9 obese mice were used. Eye-opening was recorded in 57 non-obese and 22 obese mice.

Scoring of righting: return to normal position: no reaction = 0, within 60 sec = 2, within 30 sec = 4, within 5 sec = 6, within 1 sec = 8.

Scoring of negative geotaxis, postural flexion and eye-opening is graded.

For example eye-opening: both eyes closed = 0, one eye partially open = 2, one eye fully open or 2 eyes partially open = 4, one eye fully open and one eye partially open = 6, both eyes fully open = 8.

Scoring of pivoting: locomotory activity during a 5 min period: no pivoting observed = 0, little pivoting = 2, half of the time pivoting = 4, much pivoting = 6, only pivoting = 8.

Scoring of straight line walking: observations over a 5 min period.

Scoring similar to pivoting.

open circles = non-obese, filled circles = obese.

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Table 1.

<u>Intake of solid food</u>

	5 weeks	5 months
obese	6.2 ± 0.7 (6)*	6.4 ± 0.9 (14)*
non-obese	4.3 ± 0.3 (6)	5.5 ± 0.3 (16)
hyperphagia	44%	16%
efficiency of weight gain at 5 weeks; obese 18.8 ± 2.5, non-obese 12.7 ± 0.8		

intake expressed as gr/day

the mean and the SEM are given with the number of observations in parentheses

efficiency is defined as $\dfrac{\text{weight increase per day}}{\text{food intake per day}} \times 100\%$

determined during a 3 day period
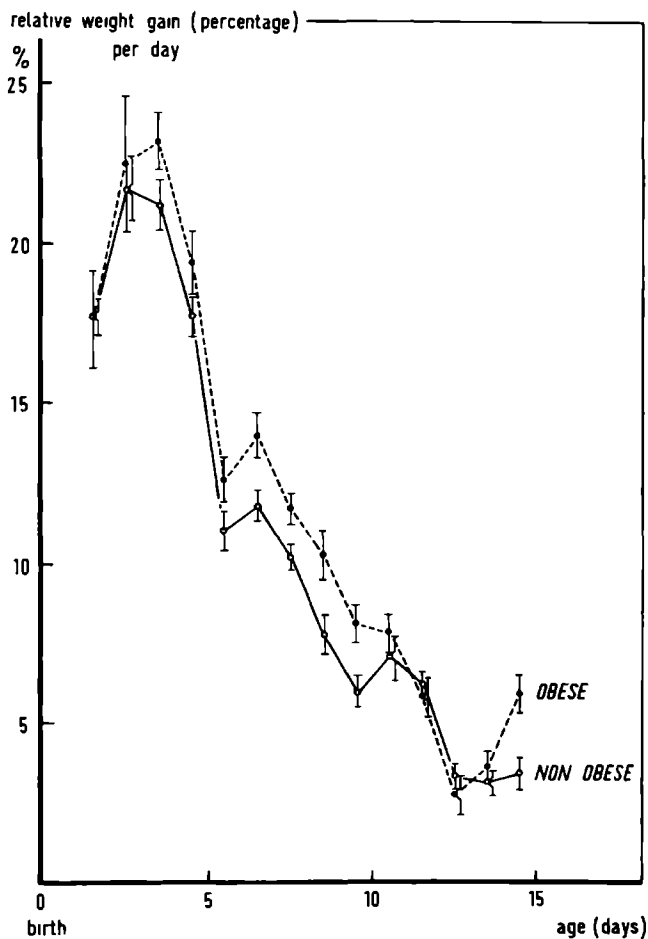
*significant at $P < 0.01$ (Student t-test)

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Fig. 3. The relative weight gain:

$(\dfrac{\text{weight on day}_{n+1} - \text{weight on day}_n}{\text{weight on day}_n} \times 100\%)$ of 26 obese and

65 non-obese. Observations include male and female mice because their respective growth patterns were similar. The mean and the SEM are indicated.

Discussion

Our results on locomotory activity show that obese mice are
less active than non-obese mice from the beginning of the
second week on, when the ability to straight-line walking
is developing to its full extent. This indicates that obese
mice are less active long before they show overt obesity at
about 4 weeks of age. This supports the idea of Mayer (5)
based on studies of locomotory activity of young adult obese
and non-obese mice of about equal weight, which indicated
that the decreased activity of obese mice can contribute to
the development of obesity. However, Yen and Acton (6),
working also with young adult mice, came to an opposite con-
clusion; inactivity being a consequence rather than a cause
of obesity. Their conclusion is based on the fact that de-
creased activity was not detectable before the animals were
overt obese. On account of the present data, it seems that
the inactivity of obese mice does not result from obesity
itself, but from the genetic condition leading to obesity.
This suggestion finds support from the data recently obtained
by Clark and Gay (12) indicating that obese mice with body
weights similar to non-obese mice as a consequence of food
deprivation, show significantly lower locomotory activity than
non-obese mice of the same age.
It could be argued, however, that a certain level of obesity
is present from day 13, since from this moment on hypertrophy
of the adipocytes of the epididymal fat body can be observed
(13). On the other hand, even though this locally developing
obesity may contribute to a minor increase in body weight, it
could by no means account for the observed weight difference
between obese and non-obese mice at this age (the difference
in weight between obese and non-obese mice at day 13 is 5%)
nor for the 50% lower activity. It may be concluded therefore,

that the low activity of obese pups is not caused by
"mechanically disadvantageous" adipose tissue.
No obvious differences in the development of reflexes between
obese and non-obese mice could be detected. As in normal
mice, the process of eye-opening begins on day 12 and is
completed at day 18 in obese mice. It, therefore, seems that
the hypothyroidism which is probably congenital, does not
interfere with the reflexological development and eye-opening.
The high growth rate of neonatal obese mice (fig. 3) can only
be explained from a more positive caloric balance, i.e. from
an increased caloric intake and/or a lower caloric expenditure.
The most important factors determining caloric expense are the
basal metabolism and physical activity. During the first post-
natal week, caloric expense can hardly differ between obese and
non-obese mice because the early postnatal mouse is poikilother-
mic. The variation in metabolic rate with environmental tempera-
ture should be equal for both types of mice which are reared
under the same conditions. Furthermore, physical activity is low,
so that possible differences would contribute little to the
difference in caloric balance. Consequently, the cause of the
high growth rate of obese mice during the first postnatal week
should be sought in the caloric intake, i.e. milk intake. It,
therefore, could be suggested that obese mice are congenitally
hyperphagic. At the end of the second week, factors determining
the caloric expenditure begin to contribute to the difference in
caloric balance. With increasing ability to perform straight-line
walking (fig. 2), total activity increases (fig. 1). However,
because obese mice are less active they should spend less calories.
Moreover, at about the same time homoiothermia will develop as is
reflected by an increasing body temperature reaching the adult
level at about 3 weeks of age. It has been established (4) that
during the period in which homeothermia develops, as well as during
adult life, the body temperature of obese mice is lower than in
normal mice, indicating a lower basal metabolic rate. Lower activity
and lower basal metabolic rate keep the caloric expenditure of obese

mice low and could make more calories available for body weight increase and enlargement of fat depots.

From the third week on, the same factors cause an extremely positive caloric balance of obese mice. The contribution of hyperphagia has now become more important (table 1), because the infant mice have started to eat solid food which is available ad lib, in contrast to milk for which they have to compete.

On the basis of the evidence available at the present the following hypothesis for the development of the obese-hyperglycemic syndrome may be suggested: the genetically obese mouse is congenitally hypothyroid and hyperphagic. In combination with the relative inactivity, this leads to a very positive caloric balance which results in obesity, followed by insulin resistance, hyperinsulinemia and later hyperglycemia and glucosuria. Each of these defects can be of hypothalamic origin. The primary cause of the syndrome could be centrally located as well.

References

1. Irgalls, A.M., Dickie, M.M. and Snell, G.D.: Obese, a new
   mutation in the house mouse. J. Heredity 41; 317, 1950.
2. Bray, G.A. and York, D.A.: Genetically transmitted obesi-
   ty in rodents. Physiol. Rev. 51; 598, 1971.
3. Van der Kroon, P.H.W.: Hereditaire obesitas by muizen.
   Dissertation, Nijmegen, The Netherlands, 1966.
4. Joosten, H.F.P.: The role of the thyroid in the develop-
   ment of the obese-hyperglycemic syndrome in mice (ob/ob).
   Metabolism, accepted for publication, 1973.
5. Mayer, J.: Decreased activity and energy balance in the
   hereditary obesity-diabetes syndrome of mice. Science 117;
   504, 1953.
6. Yen, T.T.T. and Acton, J.M.: Locomotor activity of various
   types of genetically obese mice. Proc.Soc.exp.Biol.Med. 140;
   647, 1972.
7. Eayrs J.T.: Influence of the thyroid on the central ner-
   vous system. Britt.Med.Bull. 16; 122, 1960.
8. Schapiro, S.: Metabolic and maturational effects of thyrox-
   ine on the infant rat. Endocrinol. 78; 527, 1966.
9. Fox, M.W.: Reflex ontogeny and behavioral development of the
   mouse. Anim. Behav. 13; 234, 1965.
10. Van Abeelen, J.H.F. and Kalkhoven, J.Th.R.: Behavioural on-
    togeny of the Nijmegen Waltzer, a neurological mutant in the
    mouse. Anim. Behav. 18; 711, 1970.
11. Van Eeden, C. and Rümke, Chr.L.: Wilcoxon's two sample test.
    Statistica Neerlandica 12; 275, 1958.
12. Clark, L.D. and Gay, P.E.: Activity and body-weight relation-
    ships in genetically obese animals. Biol. Psych. 4; 247, 1972.
13. Joosten, H.F.P. and Van der Kroon, P.H.W.: The enlargement of
    epididymal adipocytes in relation to hyperinsulinemia in
    obese hyperglycemic mice (ob/ob). Metabolism 23, 59, 1974.

Chapter V.

Development of the obese-hyperglycemic
syndrome in mice with a growth hormone
deficiency.

Summary

By mating mice heterozygous for the recessive gene obese $\frac{ob}{+} \frac{+}{+}$ with mice homozygous for the recessive gene dwarf $\frac{+}{+} \frac{dw}{dw}$ and subsequent mating of the offspring, mice homozygous for both the obese and dwarf gene were obtained. It was established that the genes for obese and dwarf belong to different linkage groups.

The homozygous obese dwarf mice develop obesity and hyperinsulinemia. The degree of hyperglycemia developed by these homozygotes is not significantly different from non-obese dwarf mice. Because homozygous dwarf mice are deficient in growth hormone production, it was concluded that obesity and hyperinsulinemia can develop under conditions of extreme growth hormone deficiency.

Introduction

The most predominant characteristics of the hereditary obese-hyperglycemic syndrome in mice $\left(\frac{ob}{ob}\right)$ are, extreme obesity, hyperglycemia, hyperinsulinemia, insulin resistance, hyperphagia, (1, 2) and hypothyroidism (3). It was previously found that the development of hyperinsulinemia preceeds that of obesity (4). Indications for abnormal development in $\frac{ob}{ob}$ mice become detectable soon after birth (5), however, the primary metabolic defect resulting from the homozygous $\frac{ob}{ob}$ condition has not been established.

It is well established that growth hormone (somatotropic hormone) can be responsible for the development of certain features which are also observed in obese-hyperglycemic mice. Injections of growth hormone can result in insulin resistance, hypertrophy and hyperplasia of the islands of Langerhans (6, 7) Rats with a growth hormone producing tumor show hyperinsulinemia and an increased sensitivity if the islands to glucose stimulation (8). Moreover, the impaired sensitivity of insulin

secretion of islands from hypophysectomized rats to glucose stimulation in vitro, can be restored to normal by injections of growth hormone (9).

Recently, Herbai et al. (10) suggested that an increased production of growth hormone might be involved in the development of the obese-hyperglycemic syndrome in mice. On account of these data, it appeared significant to establish whether or not growth hormone plays a key role in the development of the syndrome. In order to obtain conclusive evidence about this possible role of growth hormone, mice with a genetic constitution for obesity $\frac{ob}{ob}$ and growth hormone deficiency $\frac{dw}{dw}$ were used. Because the Snell dwarf mouse is genetically growth hormone deficient, obese dwarf mice can be obtained by combining the genetical constitution for obesity and dwarfism in one individual (genotype $\frac{ob}{ob} \frac{dw}{dw}$).

Because the linkage data concerning the genes obese and dwarf, as available in the literature, are not conclusive, linkage being concluded by van der Kroon and Buis (11), but unexpected on the basis of linkage studies by Green and Lane (12), this problem was also investigated.

Material and methods

Experimental animals

The animals were raised under constant environmental conditions; temperature $20^{\circ}C$, relative humidity 70%, artificial daylight with 13 hr light and 11 hr dark, ventilation of the animal room 15 times per hour. Water and food were supplied ad libitum.

Breeding of obese dwarf mice

Breeding of homozygous obese dwarf mice was started by mating mice heterozygous for the obese gene with mice homozygous for the dwarf gene (fig. 1).

Fig. 1.

Breeding scheme for the production of obese dwarf mice
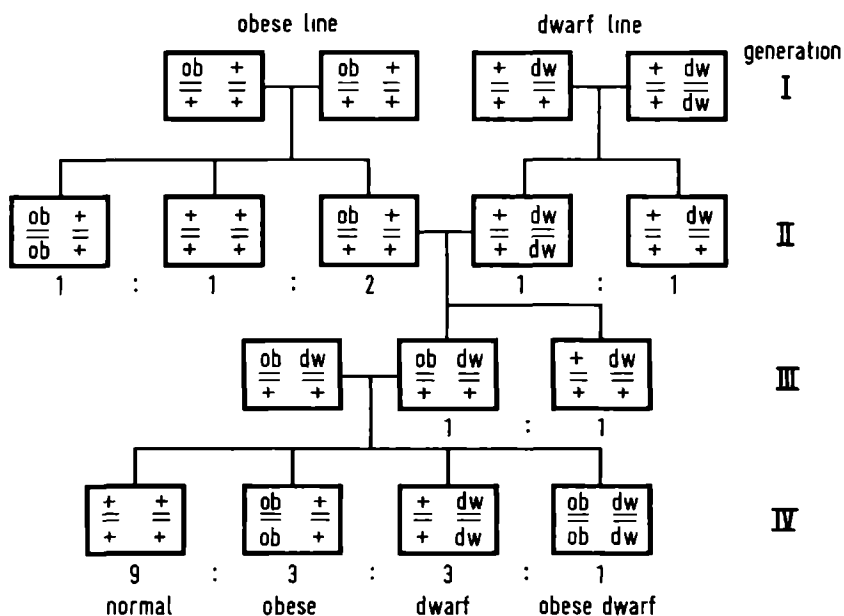
The $\frac{+}{+} \frac{dw}{dw}$ animals are normally sterile, but males of this type were made fertile by daily subcutareous transplantation of a pituitary from a normal male mouse.

The $\frac{ob}{+} \frac{+}{+}$ animals of generation II were identified by back-crossing to the parental genotype $\frac{ob}{+} \frac{+}{+}$. Animals of generation III were identified accordingly.

Since homozygous obese mice are sterile, the obese line must
be maintained by matings between heterozygous parents. The
offspring of these matings consists of mice homozygous for
the ob allele, mice homozygous for the + allele and heterozy-
gotes in a ratio of 1 : 1 : 2 (generation II, fig. 1). Since
the obese gene is recessive, heterozygous animals are pheno-
typically normal and can be identified only by progeny testing.
Homozygous dwarf mice are also sterile. However, fertility of
the male can be efficiently restored by a daily, subcutaneous
transplantation of a pituitary from a normal male mouse (Van der
Kroon and Buis, pers. communication). The dwarf line is main-
tained by mating heterozygous dwarf females with fertile homo-
zygous dwarf males. These matings result in an offspring consist-
ing of mice homozygous for dw and mice heterozygous for dw with
a ratio of 1 : 1.
In order to produce obese dwarf mice, females heterozygous for
obese $\frac{ob}{+}$ were mated with males homozygous for dwarf $\frac{dw}{dw}$. The
resulting offspring (generation III, fig. 1) consists of animals
heterozygous for dwarf, half of which are heterozygous for obese
and half of which are homozygous for the wild type gene. The two
phenotypically normal types can be distinguished by backcrossing
to animals heterozygous for obese. Only those parents, which produce
obese individuals among their progeny were heterozygous for both
obese and dwarf, and these mice were mated to each other.
A total number of 46 matings of this type produced an offspring of
414 mice. A number of these mice (generation IV, fig. 1) were
taken for determinations of body weight, blood glucose and plasma
insulin.

Methods

Plasma insulin was determined by a radioimmunoassay with
a kit available from the Radiochemical Centre, Amersham,
using purified ox insulin as a standard. Blood was obtained
from the orbital plexus with heparinized 50 µl glass
capilaries. Blood samples were diluted three fold with a
buffered saline-albumin solution and centrifuged in micro-
tubes. 0.1 ml samples of the diluted plasma were used for
the assay.

Blood glucose was determined enzymatically (GOD method,
Boehringer) after deproteinization with 0.16% uranyl acetate
in 0.9% saline.

In order to assay for the presence of GH (growth hormone) in
the experimental animals, disc electrophoresis was performed
with homogenates of pituitaries prepared in Tris-HCl buffer
pH 7.3 containing 0.7 M sucrose (13). Pituitaries were rinsed
in saline and homogenized with a Potter homogenizer of 1 ml
capacity. The homogenates were placed directly on top of the
separation gel. Samples prepared from non-dwarf mice were
equivalent to a homogenate of 0.1 pituitary and samples from
dwarf mice equivalent to 1.1 pituitary. After electrophoresis
for 2 hrs at 2 mA in 7% acrylamide gels in Tris-HCl buffer,
pH 8.9, the gels were stained with amido black, differentiated
by immersion in a 7% acetic acid solution and scanned densito-
metrically on a Zeiss densitometer.

The matings of the double heterozygotes $\frac{ob}{+}\frac{dw}{+} \times \frac{ob}{+}\frac{dw}{+}$
gave an offspring of 414 mice (table 1). Among this off-
spring, there were 4 types of mice: normal mice, obese
mice, dwarf mice and obese dwarf mice. Because of the
variability of the expression of obesity in dwarf ani-
mals, some animals (in this experiment 4) were difficult
to classify either as obese dwarfs or non—obese dwarfs.
Even though the distinction was made on the basis of body
weight comparisons rather than on the phenotype of mice
of the same litter, these 4 mice remained unclassifiable.
If there were no linkage between the obese and dwarf genes,
a segregation ratio of 9 : 3 : 3 : 1 is expected. On the
other hand, if the genes were located on the same linkage
group, a significant deviation from this ratio, which
corresponds to a 233 : 78 : 78 : 26 ratio (total number of
animals: 414) should be found. The number of obese dwarfs
should be in the order of $\frac{1}{16}$ of the offspring. The number of
obese dwarf mice actually found among the offspring was
between 20 and 24, the uncertainty being due to the afore-
mentioned difficulty in the classification of 4 dwarf animals.
The segregation data were tested for linkage following
Fisher (14) for both extremes. Even if we would assume that
the 4 problem cases were non—obese dwarfs, linkage between the
genes obese and dwarf would be extremely unlikely $(0.50 < P
< 0.90)$.
In order to make certain that the obese dwarf and non—obese
dwarf mice fail to produce GH, a comparison of the protein
pattern of pituitary homogenates was made by means of disc-
electrophoresis in acrylamide gels. Densitometric tracings of
amido black stained gels reveal that in pituitary homogenates
of normal mice and obese non—dwarf mice a very pronounced
protein band is visible, which is absent in tracings of obese
dwarf and non—obese dwarf mice (fig. 2).

Table 1.

Segregation data of matings $\frac{ob}{+} \frac{dw}{+}$ X $\frac{ob}{+} \frac{dw}{+}$

Phenotype	dwarf	non-dwarf	total
obese	20 / 24 (26)	72 (78)	92 / 96 (104)
non-obese	81 / 77 (78)	241 (233)	322 / 318 (311)
total	101 (104)	313 (311)	414

An offspring of 414 mice was obtained from 46 matings.
There were 4 dwarf animals of which it was uncertain whether
they belonged to the obese dwarf group or to the non-obese
dwarf group.

The expected number of animals of the 4 possible genotypes
was calculated on the basis of a 9 : 3 : 3 : 1 segregation
ratio, assuming an independent segregation of the two genes.
This expected number is given in parentheses.

Fig. 2.

Densitometric tracings of amido black stained acrylamide
gels prepared from pituitary homogenates from normal, obese,
obese dwarf and dwarf mice.
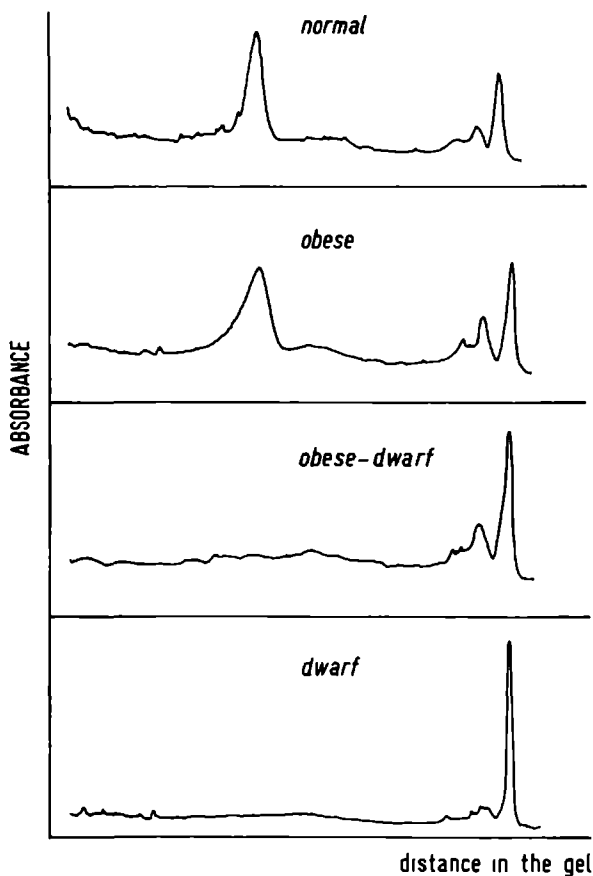
Electrophoresis was applied to whole homogenates. Samples
prepared from normal and obese mice contained an equivalent
of 0.1 pituitary, and samples from obese dwarf and dwarf
mice contained an equivalent of 1.1 pituitary.

The absence of this band cannot be the result of a differ-
ence in loading of the gels since the equivalent number
of pituitaries used for GH determination in dwarf homo-
genates is 11 times higher than in the non-dwarf homo-
genates.

A comparison of the following features; blood glucose,
plasma insulin and body weight indicated that, in spite
of the absence of GH, obesity and hyperinsulinemia develop
(table 2).

Both types of obese mice have an overweight of 60-70% as com-
pared to their respective controls. The variability of body
weight of obese-dwarf mice is much higher than of non-obese
dwarf mice. Hyperinsulinemia is very well expressed in obese
dwarf mice, however, the degree is lower than in obese non-
dwarf mice. Hyperglycemia is absent in obese dwarf mice, al-
though a tendency for increased blood glucose values in obese
dwarfs as compared with non-obese dwarfs can be observed when
littermates are compared.

Table 2

Body weight, plasma insulin and blood glucose of normal, obese, dwarf and obese dwarf mice

	nor-obese nor-dwarf		obese non-dwarf		non-obese dwarf		obese dwarf	
	♀♀		♀♀		♀♀		♀♀	
body weight (gr)	21.8 + 0.5 (10)	24.4 + 0.8 (10)	37.4 + 1.2 (7)	38.3 + 1.3 (8)	7.2 + 0.4 (8)	6.3 + 0.2 (9)	12.0 + 1.2 (7)	10.7 + 0.6 (7)
plasma insulin (μJ/ml)	26.1 + 7.9 (9)	25.9 + 6.8 (8)	326.9 + 46.6 (7)	232.0 + 44.9 (8)	14.5 + 3.6 (6)	28.3 + 6.7 (9)	106.7 + 15.9 (7)	61.3 + 16.0 (7)
blood glucose (mg%)	96.2 + 4.5 (10)	91.8 + 3.9 (9)	114.4 + 5.7 (7)	196.8 + 29.5 (8)	66.4 + 7.0 (7)	52.8 + 4.7 (8)	70.0 + 8.6 (7)	70.5 + 8.0 (7)

Obese dwarfs have a higher bodyweight and higher insulin levels as compared to

non-obese dwarfs (P< 0.01).

Blood glucose concentration is not significantly increased.

The mean and SEM are indicated.

Number of observations in parentheses.

Fig. 3.

Adiposity of the obese dwarf mouse

Obese dwarf on the left and non-obese dearf on the right.
The abdominal cavity is opened to show the excessive fat
deposition in obese dwarfs as compared to non-obese dwarfs.

On the basis of the finding that obese mice have a high
sulphate incorporation into costal cartilage, Herbai et
al. (10) suggested that obese mice have an increased
level of plasma GH. This increased GH level was con-
sidered as a possible cause for the development of the
obese-hyperglycemic syndrome of these mice. However,
this bioassay of GH is not reliable in obese mice because
it is well known that also insulin can stimulate the in-
corporation of sulphate into costal cartilage of mice (10,
see also 15). Because obese mice display hyperinsulinemia,
it is at least, difficult to ascribe a higher incorporation
of sulphate to an increased GH liter.
By making the appropriate crosses between a dwarf strain and
an obese strain, a number of mice was produced in which the
genetical constitutions for obesity and dwarfism were com-
bined. The dwarfism of these mice, inherited from the Snell
dwarf strain, is caused by a primary GH deficiency as a
result of a pituitary insufficiency (16). GH is not detectable
in plasma of Snell dwarf mice (17). Our mice, homozygous for
obesity $\frac{ob}{ob}$ and dwarfism $\frac{dw}{dw}$, did develop obesity and
dwarfism. Yanai et al. (18) and Lewis et al. (19) have shown
that the major protein band in gels ran with pituitary homo-
genates of non-dwarf mice is identical with GH. This bard is
absent in the electropherograms from non-obese dwarf and
obese dwarf mice, indicating that these mice do not have sig-
nificant quantities of GH made. The essential features of
the obese-hyperglycemic syndrome, obesity and hyperinsulinemia
are expressed in obese dwarfs. Obese dwarf mice have an over-
weight of 60-70% as compared to non-obese dwarfs and adiposity
is clearly visible (fig. 3).

Hyperinsulinemia is present, but not as extreme as in obese non-dwarf mice (table 2). The lower degree of hyperinsulinemia could be explained from a deficient development of tꞋe islands of Langerhans in obese dwarfs. It is known (20) that after weaning, cell division stops in dwarf mice with as a consequence that hyperplasia and hypertrophy of islands of Langerhans can hardly develop. In contrast to obese non-dwarfs, hyperglycemia could not be detected in obese dwarfs, although comparisons within litters indicated a tendency for increased blood glucose levels to be present.

From the data presented, it may be inferred that in spite of the virtual absence of GH in the obese dwarf mouse $\frac{ob}{ob}\frac{dw}{dw}$, the characteristic syndrome of the obese hyper-glycemic mouse develops. Therefore, GH can not be essential for the development of this syndrome in these mice. Consequently, an increased circulating level of GH in the obese non-dwarf mouse, if present, could only contribute and aggrevate the syndrome.

Abnormal plasma GH levels and pituitary GH content have been reported for several genetically obese and diabetic strains of mice. In the mutant mouse diabetes (db) (21) and in the polygenic KK mouse (22) strong indications for hypersecretion of GH have been found. In the NZO (New Zealand Obese) mouse, however, plasma GH concentration is normal (23). The GTG (GoldThioGlucose) obese mouse has subnormal plasma GH concentration (24). On account of these reports it may be concluded that in various strains developing diabetes and/or obesity the plasma GH level can be normal, subnormal or over-normal. This indicates that if GH is involved in the development of these syndromes, it is certainly not the primary cause.

The conclusion of Green and Lane (12) that the genes
for obesity (ob) and for dwarfism (dw) are not linked
is justified by the present linkage data which show
an independent segregation of ob and dw, as can be
concluded from the observed 9 : 3 : 3 : 1 ratio. Be-
cause it is well established that the obese gene
belongs to linkage group VI, the linkage group to
which the dwarf gene belongs remains to be established.

Acknowledgement

References

1. Bray, G.A. and York, D.A.: Genetically transmitted obesity in Rodents. Physiol. Rev. 51, 598 (1971).

2. Stauffacher, W., Orci, L., Cameron, D.P., Burr, I.M. and Renold, A.E.: Spontaneous hyperglycemia and/or obesity in laboratory rodents: an example of the possible usefulness of animal disease models with both genetic and environmental components. Rec. Progr. Horm. Res. 27, 41 (1971).

3. Joosten, H.F.P. and Van der Kroon, P.H.W.: The role of the thyroid in the development of the obese-hyperglycemic syndrome in mice (ob/ob). Metabolism, in press.

4. Joosten, H.F.P. and Van der Kroon, P.H.W.: The enlargement of epididymal adipocytes in relation to hyperinsulinemia in obese-hyperglycemic mice ($\frac{ob}{ob}$). Metabolism 23, 59, 1974.

5. Joosten, H.F.P. and Van der Kroon, P.H.W.: Physiological and behavioral treats associated with the development of the obese-hyperglycemic syndrome in mice (ob/ob). Metabolism, in press.

6. Ogilvie, R.F.: The endocrine pancreas in human and experimental diabetes. Ciba Foundation, Coll. Endocrinol. 15, 49 (1964).

7. Mahler, R., Szabo, O., Adler, K. and Levine, R.: The role of an intact pancreas in the production of insulin resistance by growth hormone. Fed. Proc. 29, 380 (1970).

8. Martin, J.M., Akerblom, H.K. and Garay, G.: Insulin secretion in rats with elevated levels of circulating growth hormone due to MtT-W15 tumor. Diabetes 17, 661 (1968).

9. Malaisse, W.J., Malaisse-Lague, F., King, S. and Wright, P.H.: Effect of growth hormone on insulin secretion. Amer. J. Physiol. 215, 432 (1968).

10. Herbai, G., Westman, S. and Hellerström, C.: The growth hormone dependent incorporation of sulphate into the costal cartilage of obese-hyperglycaemic mice of different ages. Acta Endocrinol. <u>64</u>, 415 (1970).

11. Van der Kroon, P.H.W. and Buis, A.J.M.: Linkage of dwarf with obese in the mouse. Genetica <u>41</u>, 57 (1970).

12. Green, M.C. and Lane, P.W.: Mutant genes and linkages. 42nd annual report 1970-1971 of the Jackson Laboratory, p. 55, (1971).

13. Maurer, H.R.: Disc Electrophoresis. W. de Gruyter, Berlin (1971).

14. Fisher, R.A.: A preliminary linkage test with agouti and undulated mice. Heredity <u>3</u>, 229 (1949).

15. Laron, Z., Karp, M., Pertzelan, A. and Kauli, R.: Insulin, growth and growth hormone. Israel J. Med. Sci. <u>8</u>, 440 (1972).

16. Elftman, H. and Wegelius, O.: Aterior pituitary cytology of the dwarf mouse. Anat. Rec. <u>135</u>, 43 (1959).

17. Garcia, J.F. and Geschwind, I.I.: Investigation of growth hormone secretion in selected mammalian species. Growth Hormone. Eds.: Pecile, A. and Müller, E.E. p. 267. Excerpta Medica Foundation, Int. Congress, Amsterdam (1968).

18. Janai, R., Nagasawa, H. and Ruretain, K: Disc electrophoretic analysis of growth hormone and prolactin in the anteroir pituitary of mice. Endocrinol. Japan <u>15</u>, 365, (1968).

19. Lewis, M.J., Cheever, E.V. and Van der Laan, W.G.: Studies on the growth hormone of normal and dwarf mice. Endocrinol. <u>76</u>, 210, (1965).

20. Winick, M. and Grant, P.: Cellular growth in the organs of the hypopituitary dwarf mouse. Endocrinol. <u>83</u>, 544 (1968).

21. Desjardins, C.: Pituitary growth hormone and its hypothalamic releasing factor in normal and genetically diabetic mice. Proc. Soc. Exp. Biol. Med. <u>130</u>, 1 (1969).

22. Nakamura, M.: Cytological and histological studies on the pancreatic islets of a diabetic strain of the mouse. Z. Zellforsch. <u>65</u>, 340 (1965).

23. Larkins, R.G.: Plasma growth hormone in the New Zealand Obese Mouse. Diabetologia <u>7</u>, 302 (1971).

24. Müller, E.E., Miedico, D., Giustina, G., Pecile, A., Cocchi, D. and Mandelli, V.: Impaired secretion of growth hormone in Goldthioglucose Obese Mice. Endocrinol. <u>89</u>, 56 (1971).

Chapter VI

Discussion

The results of the present investigation are still insuffi-
cient to provide a clear answer to the question as to what
should be considered the primary defect of the obese-hyper-
glycemic syndrome in genetically obese mice. On the other
hand, these results provide a solid basis for further studies
aiming at the solution of this problem. A survey of the avail-
able data, including the sequence in which the various featu-
res of the obese-hyperglycemic syndrome become manifest du-
ring the early development, may give clues as to the direction
in which forthcoming research should be pointed.
The rapid growth of adipose tissue becomes evident at a very
early stage in the development of the syndrome. Because fat
cell diameters in obese and non-obese mice can be distin-
guished from the 14th day of development, it seems likely
that a difference in either lipogenesis and/or lipolysis be-
tween obese and non-obese mice does occur before this time.

Since hyperinsulinemia and hyperglycemia develop later, it
seems reasonable to suggest that obesity, rather than diabe-
tes, is the primary defect or a direct consequence thereof.
It can be excluded that obesity is a consequence of hyper-
insulinemia on account of the plasma insulin data and on
the basis of submicroscopic studies of the islands of Langer-
hans. The latter study revealed that no clear indications
for differences in beta cell activity can be observed in
islands of 14 day old obese and non-obese mice (unpublished
observations).

A substantial number of abnormal characteristics of the gene-
tically obese mouse could be explained or the basis of a hypo-
thyroidism. Although it seemed well established that obese
mice were euthyroid (45, 46), the present results clearly

indicate hypothyroidism of obese mice older than 4 weeks.
Measurements of the body temperature indicated that hypo-
thyroidism may occur as early as the 10th day of postnatal
development. Moreover, the lates results from a study of
heart beat frequency and locomotor activity revealed a
clearly lower level of activity in obese as compared to non-
obese mice, even during the first week of postnatal develop-
ment. Also these features may be seen as a consequence of hy-
pothyroidism in obese mice. So far, it remained uncertain
whether or not hypothyroidism is a congenital defect.

The low level of muscular activity, heart beat frequency and
locomotor activity, could explain in part, the observed high
rate of body weight increase of obese mice as compared to
non-obese mice. It seems unlikely, however, that on the basis
of the low muscular activity alone sufficient calories could
be saved to support the excessive growth. It, therefore, may
be expected that in addition to the energy saving by low mus-
cular activity, the high growth rate is supported by a conge-
nitally higher caloric intake ; i.e. the obese mouse could be
congenitally nyperphagic. Hyperphagia is a well established
abnormality of the postweaning obese mouse, but food intake
during the first week of postnatal life has never been studied.

The data obtained from the experiments described in this dis-
sertation suggest that the obese-hyperglycemic syndrome in
mice originates from a congenital hypothyroidism and a con-
genital hyperphagia, phenomena which could both result from
a common hypothalamic defect. As may become evident from the
following discussion, this hypothesis finds more support
from the data presented than previous ideas concerning the
origin of the syndrome.

In 1963, Treble and Mayer (31) suggested that the obese-hyper-
glycemic syndrome develops in the $\frac{ob}{ob}$ mouse as a consequence of

an enzymopathy. This suggestion was based on his finding that the enzyme glycerol kinase, which was thought to be absent from adipose tissue of normal mice, is present in adipose tissue of obese mice. This enzyme controls the formation of alpha glycerophosphate from glycerol and ATP. Alpha glycerophosphate is the acyl-CoA acceptor in the metabolic pathway leading from glycerol to triglyceride. The presence of this enzyme in adipose tissue of obese mice would have as a possible consequence that glycerol, one of the products of lipolysis in adipose tissue, does not leave the adipocyte but is used immediately as a substrate for lipogenesis. This could explain the accumulation of lipids in adipocytes of obese mice. However, data more recently obtained by Koschinsky et al. (47, 48, 49) indicate that glycerol kinase is not restricted to adipose tissue of obese mice, but is also found in adipose tissue of non-obese mice. Other data reported by Koschinsky et al. abtained from a study of the relation between plasma insulin concentration and specific activity of adipose tissue glycerol kinase indicate that insulin can regulate the glycerol kinase activity of adipose tissue. These observations indicate that this enzyme can not play a primary role in the development of the syndrome because it was shown that plasma insulin of obese mice is not increased as compared to non-obese mice before the age of 3 weeks, whereas clear indications for obesity can be observed at an age of 2 weeks.

On the basis of determinations of the in vivo incorporation of (^{32}S) sulphate in costal cartilage of obese mice, Herbai, Westman and Hellerström (42, 43) suggested that the quantity of plasma growth hormone was increased. However, GH producing alpha cells in the pituitary of obese mice did not show any sign of an increased activity when studied electronmicroscopically (44). Moreover, it is well established that high concentrations of insulin can stimulate the incorporation of sulphate into cartilage (50). Therefore, it is not excluded

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that the higher [32]Sulphate incorporation in costal cartilage observed in obese mice results from the high plasma insulin level, rather than from a high GH titer. The possibility that GH plays an essential role in the pathogenesis of the obese-hyperglycemic syndrome can be excluded on the basis of data reported in this thesis which indicate that the syndrome can develop in the absence of endogeneous GH in the case of the obese dwarf mouse.

In 1969, Stauffacher and Renold discovered that diaphragm tissue of their obese mice was not sensitive to insulin (32, 33, 51), whereas adipose tissue was sensitive. This finding could be of essential significance because it would provide an explanation for the rapid growth of adipose tissue of obese mice. Unfortunately however, the insulin insensitivity of muscle tissue is reversible and the detection of it is dependent upon the age of the animal under investigation. Insulin sensitivity of muscle tissue reappears after norma-lization of body weight (52). Because there are strong indi-cations that muscle tissue of preweaning obese mice has a normal insulin sensitivity (35), even though these mice already display signs of obesity (this thesis) it seems obvi-ous that muscle insulin insensitivity could hardly be consi-dered as the primary genetic defect in obese mice.

A supposed hypersensitivity of the beta cells of the islands of Langerhans to carbohydrate stimulation does play a central role in the hypothesis of Genuth et al. (35, 53). This hy-pothesis is based on the observation that obese mice, from 4 to 6 weeks of age, have a high plasma insulin concentration in spite of a normal blood glucose concentration. However, this hypothetical hypersensitivity of beta cells could not be prooven in a study of Lernmark and Hellman (54) on the insulin secretion response of isolated islands of adult obese mice to the stimulating action of glucose. In fact,

there is no clear evidence for this hypothetical hypersensi-
tivity of individual islands of obese mice. So it seems, that
hypersensitivity of the islands to carbohydrate stimulation
can hardly be considered as an explanation for the observed
hyperinsulinemia. Besides, data reported in this thesis show
that hyperinsulinemia is a relatively late phenomenon in the
development of the syndrome.

Chlouverakis et al. (34, 39) consider adiposity as the first
detectable phenomenon of the syndrome. In their view, hyper-
insulinemia and insulin resistance are secondary events. Our
results on the growth of the epididymal adipose tissue sup-
ports this concept. Ideas about the mechanism bringing avout
adiposity of obese mice were not reported by these authors.

Strautz (36, 37) reported some very interesting experiments
which led him to suggest that a, so far hypothetical, factor
is missing from the islands of Langerhans of obese mice, a
factor which should be present in the islands of normal mice.
According to this suggestion, the obese-hyperglycemic syndrome
is a deficiency syndrome. This hypothesis was based on the
results of transplantation experiments. Transplantation of
pieces of pancreas, or isolated islands from normal mice
into obese mice effectively prevented the development of
overweight, hyperglycemia and hyperinsulinemia. In his opi-
nion, these results support the idea of the presence of a
factor in the islands of normal mice that could act as a
repressor of adipose tissue glycerol kinase. In this way,
Strautz explains the adipose tissue glycerol kinase activity
of obese mice, and thus the development of obesity. Although
for reasons previously discussed, the second part of this
hypothesis can be disregarded. The suggestion that there
may be a factor present in non-obese islands which is absent
from obese islands is attractive.
Since the transplantation experiments are of crucial signi-
ficance for this suggestion, and no other data supporting

the results of Strautz are available, an attempt was made
to repeat these experiments in our laboratory. Although the
isolated islands of Langerhans showed normal insulin secre-
tion kinetics in vitro, they were not viable after tranplan-
tation into the acceptor animals. Other laboratories were
also unsuccesful in their efforts (Bray, personal communi-
cation). A possible explanation for the failure to confirm
the results of Strautz may be found in differences in immu-
nological characteristics between the islands of the donor
mice and the host, causing rapid degeneration of the implan-
ted islands. Even though our obese strain is an inbred strain,
and it was therefore expected that immunological differences
between individuals should be minimal, some heterogeneity
couls still be present in the genetic background responsible
for the tissue-immnumological properties. On the other hand,
even if the experiments of Strautz could be repeated, the
data would not necessarily support the conclusion, that the
primary genetic defect is located in the pancreatic islands.
As such, the results would not discriminate between the pos-
sibilities that the defect in the function of obese islands
is a primary consequence of the mutation, rather than an
irreversible defect, evident at 6 weeks of age, but developed
from another defect occurring earlier in the life of the
obese mouse and localized elsewhere in the body. In our view,
the problem of discriminating between the two alternatives
could be solved by transplantation of islands from very young
mice instead of those of mice of 6 weeks of age. If we assume
that there is a defect in the islands of obese mice which
is the consequence of the malfunctioning of an organ or
organsystem regulating the island function, it is very like-
ly to consider the hypothalamus as the organ in which the
primary defect has to be sought. This speculation is based on
the known relationship between the hypothalamus and the in-
sulin secretion of the islands of mice and rats, as has been
described by Idahl and Martin (55) and Martin (56). A factor,

capable of stimulating insulin secretion of isolated islands, can be isolated from the ventrolateral part of the hypothalamus of rats, but not from the ventromedial part. This factor can be isolated also from normal rat blood plasma. If the hypothalamus has, indeed, a regulatory function in insulin secretion, it is not unlikely that the hypothalamus is also active during the period of differentiation of the islands of Langerhans, and thus may play a key role in the development of the syndrome.

In conclusion, it may be pointed out that the hypothesis as developed on the basis of the results presented in this thesis agrees with those of Chlouverakis et al. in that obesity is a phenomenon closely related to the primary defect and that hyperinsulinemia, insulin resistance and hyperglycemia are secondary features of the syndrome. In Genuth's view, the supposed hypersensitivity of the beta cells of the islands of Langerhans is caused by a disturbed hypothalamic function and/or gastrointestinal function which can, or can not be coincident with hyperphagia. We agree with Genuth, that the hypothalamus could be the site of the primary genetic defect. In our hypothesis, hyperphagia is a congenital defect. A new element in the hypothesis is the suggestion of congenital hypothyroidism.

The advantage of the hypothesis here presented is that it explains a great number of defects in the etiology of the obese-hyperglycemic syndrome, and provides an attractive basis for future research.

Chapter VII

Summary

The obese-hyperglycemic syndrome in mice $\frac{ob}{ob}$ is a compli-
cated syndrome, which is characterized in its adult form
by endocrine, metabolic and behavioral anomalies.

In an effort to identify and localize the primary genetic
defect, a number of features of the syndrome such as,
hypothyroidism, hyperglycemia, hyperinsulinemia, decreased
locomotor activity, obesity and increased body growth were
investigated in order to determine the timing of their
first appearance in the course of development of the syn-
drome.

It was established that the development of this mutant
mouse differs from normal with respect to body growth from
birth on. The obese mouse is hypothyroid at least from four
weeks of age on. Data, indicating that hypothyroidism is
congenital were obtained. Of the known major anomalies of
the syndrome (obesity, hyperglycemia, hyperinsulinemia,
insulin resistance), obesity is the first anomaly that can
be distinguished.

The experiments lead to the conclusion that neither abnormal
growth hormone availability, nor hyperinsulinemia or insulin
resistance can be considered as primary defects in the
etiology of the syndrome.

A hypothesis is presented suggesting that congenital hyper-
phagia and congenital hypothyroidism, probably originating
from a hypothalamic defect, are responsible for the entire
pattern of abnormalities arising during the lifetime of the
obese-hyperglycemic mouse.

Chapter VIII

Samenvatting

Het obesitas-diabetes syndroom in de muis $\frac{ob}{ob}$ is een
ingewikkeld syndroom, dat in zijn volgroeide vorm ge-
kenmerkt wordt door endocrine-, metabole- en gedrags-
afwijkingen. In een poging tot identifikatie en lokali-
satie van het primaire genetische defect werden een aan-
tal eigenschappen van het syndroom zoals: hypothyroidie,
hyperglycemie, hyperinsulinemie, verlaagde locomotorische
aktiviteit, obesitas en versnelde lichaamsgroei onderzocht,
met in eerste instantie, het doel om het tijdstip vast te
stellen waarop deze eigenschappen zich voor het eerst
manifesteren tijdens de ontwikkeling van het syndroom.
Vastgesteld werd, dat de ontwikkeling van deze mutant van-
af de geboorte afwijkt voor wat betreft zijn lichaamsgroei.
De obese muis is vanaf een leeftijd van tenminste vier weken
hypothyroid. Er werden aanwijzingen verkregen voor het
congenitaal aanwezig zijn van deze hypothyroidie.

Van de reeds bekende hoofdkenmerken van het syndroom
(obesitas, hyperglycemie, hyperinsulinemie en insuline resis-
tentie) openbaart de obesitas zich het eerst.

De uit dit onderzoek verkregen gegevens leiden tot de kor-
klusie dat noch een afwijkende groeihormoon produktie, noch
de hyperinsulinemie of de insuline resistentie beschouwd
kunnen worden als het primaire genetische defect in de etio-
logie van het syndroom.

Het proefschrift bevat een hypothese ter verklaring van het
ontstaan van het syndroom, die een congenitale hyperfagie en
een congenitale hypothyroidie veronderstelt, welke beide hun
oorsprong zouden kunnen vinden in een afwijking in de hypotha-
lamus.

References

1. Sharkey, T.P.: Diabetes mellitus. Present problems and new research. J. Amer. Dietetic Ass. 58, 201, 1971.

2. Heald, F.P.: The natural history of obesity. Adv. Psychosom. Med. 7, 102, 1972.

3. Sharkey, T.P.: Diabetes mellitus. Present problems and new research. J. Amer. Dietetic Ass. 58, 336, 1971.

4. Sharkey, T.P.: Diabetes mellitus. Present problems and new research. J. Amer. Dietetic Ass. 58, 442, 1971.

5. Sharkey, T.P.: Diabetes mellitus. Present problems and new research. J. Amer. Dietetic Ass. 58, 528, 1971.

6. Bray, G.A., and York, D.A.: Genetically transmitted obesity in Rodents. Physiol. Rev. 51, 598, 1971.

7. Brunk, R.: Spontandiabetes bei Tieren, in: Dörzbach (ed.): Handbook of experimental pharmacology. Vol. 32, part 1, insulin, p. 203, 1971.

8. Renold, A.E., Cameron, D.P., Amherdt, M., Stauffacher, W., Marliss, E., Orci, L. and Rouiller, Ch.: Endocrine-metabolic anomalies in rodents with hyperglycemic syndromes of hereditary and/or environmental origin. Israel J. Med. 8, 189, 1972.

9. Danforth, C.H.: Hereditary adiposity in mice. J. Heredity 18 153, 1927.

10. Ingalls, A.M., Dickie, M.M. and Snell, G.D.: Obese, a new mutation in the house mouse. J. Heredity 41, 317, 1950.

11. Falconer, D.S. and Isaacson, J.H.: Adipose, a new inherited obesity of the mouse. J. Heredity 50, 290, 1959.

12. Hummel, K.P., Dickie, M.M. and Coleman, D.L.: Diabetes, a new mutation in the mouse. Science 153, 1127, 1966.

13. Bielschowsky, M. and Bielschowsky, F.: The New Zealand strain of obese mice. Austral. J. Exp. Biol. 34, 181, 1956.

14. Kondo, K., Nozawa, K., Tonita, T. and Ezaki, K.: Inbred strains resulting from Japanese mice. Bull. exp. Anim. 6, 107, 1957.

15. Jones, E.E.: Spontaneous hyperplasia of the pancreatic islets associated with glucosuria in hybrid mice. in: The struture and metabolism of the pancreatic islets. Brolin, S.E., Hellman, B. and Knutson, H. (Eds.). New York: Macmillan, 1964.

16. Christophe, J.: Le syndrome récessif obésité-hyperglycémie de la souris. Ses relations possibles avec le diabète gras humain. Bull. Acad. Belg. 5, 309, 1965.

17. Mayer, J., Dickie, M.M., Bates, M.W. and Vitale, J.J.: Free selection of nutrients by hereditarily obese mice. Science 113, 745, 1951.

18. Mayer, J., Bates, M.W., and Dickie, M.M.: Hereditary diabetes in genetically obese mice. Science 113, 746, 1951.

19. Bleisch, V.R., Mayer, J. and Dickie, M.M.: Familial diabetes mellitus in mice, associated with insulin resistance, obesity, and hyperplasia of the islands of Langerhans. Amer. J. Pathol. 28, 369, 1952.

20. Mayer, J., Russell, R.E., Bates, M.W. and Dickie, M.M.: Basal oxygen consumption of hereditarily obese and diabetic mice. Endocrinol. 50, 318, 1952.

21. Goldberg, R.C. and Mayer, J.: Normal iodine uptake and anoxia resistance accompanying apparent hypometabolism in the hereditarily obese- hyperglycemic syndrome. Proc. Soc. Exptl. Biol. Med. 81, 323, 1952.

22. Mayer, J. and Barrnett, R.J.: Sensitivity to cold in the hereditary obesity-diabetes syndrome of mice. Yale J. Biol. Med. 26, 38, 1953.

23. Mayer, J.: Decreased activity and energy balance in the hereditary obesity-diabtes syndrome of mice. Science 117, 504, 1953.

24. Mayer, J. and Jones, A.K.: Hypercholesteremia in the hereditary obese-hyperglycemic syndrome of mice. Amer. J. Physiol. 175, 339, 1953.

25. Bates, M.W., Zomzely, C. and Mayer, J.: Fat metabolism in three forms of experimental obesity : instantaneous rates of lipogenesis in vivo. Amer. J. Physiol. 181, 187, 1955.

26. Bates, M.W., Mayer, J. and Nauss, S.F.: Fat metabolism in
    three forms of experimental obesity. Acetate incorporation.
    Amer. J. Physiol. 180, 304, 1955.

27. Leboeuf, B., Lochaya, S., Leboeuf, N., Wood, F.C., Mayer,
    J. and Cahill, G.F.: Glucose metabolism and mobilization
    of fatty acids by adipose tissue from obese mice. Amer. J.
    Physiol. 201, 19, 1961.

28. Steinmetz, J., Lowry, L. and Yen, T.T.T.: An analysis of
    the lipolysis in vitro of obese-hyperglycemic and diabe-
    tic mice. Diabetologia 5, 373, 1969.

29. Mengel, K. and Schwabe, U.: Lipolysis by isolated fat cells
    of obese mice. (abstr.). Arch. Pharmakol. Exptl. Pathol.
    260, 176, 1968.

30. Marshall, N.B. and Engel, F.L.: Influence of epinephrine
    and fasting in adipose tissue content and release of fatty
    acids in obese-hyperglycemic and lean mice. J. Lipid Res.
    1, 339, 1960.

31. Treble, D.H. and Mayer, J.: Glycerolkinase activity in white
    adipose tissue of obese-hyperglycemic mice. Nature 200,
    363, 1963.

32. Stauffacher,W. and Renold, A.E.: Effect of insulin in vivo
    on diaphragm and adipose tissue of obese mice. Amer. J.
    Physiol. 216, 98, 1969.

33. Stauffacher, W., Lambert, A.E., Vechio, D. and Renold, A.E.:
    Measurements of insulin activities in pancreas ans serum
    of mice with spontaneous ("obese" and "New Zealand Obese")
    and induced (Goldthioglucose) obesity and hyperglycaemia
    with considerations on the pathogenesis of the spontaneous
    syndrome. Diabetologia 3, 230, 1967.

34. Chlouverakis, C. and White, P.A.: Obesity and insulin re-
    sistance in the obese-hyperglycemic mouse (ob/ob). Meta-
    bolism 18, 998, 1969.

35. Genuth, S.M., Przybylsky, R.J. and Rosenberg, D.M.: Insu-
    lin resistance in genetically obese-hyperglycemic mice.
    Endocrinol. 88, 1230, 1971.

36. Strautz, R.L.: Islet implants: reduction of glucose levels in the hereditary obese mouse. Endocrinol. <u>83</u>, 975, 1968.

37. Strautz, R.L.: Studies of hereditary obese mice (obob) after implantation of pancreatic islets in millipore filter capsules. Diabetologia <u>6</u>, 306, 1970.

38. Westman, S.: Development of the obese-hyperglycemic syndrome in mice. Diabetologia <u>4</u>, 141, 1968.

39. Chlouverakis, C., Dade, E.F. and Batt, R.A.L.: Glucose tolerance and time sequence od adiposity, hyperinsulinemia and hyperglycemia in obese-hyperglycemic mice (ob/ob). Metabolism <u>19</u>, 687, 1970.

40. Hellman, B.: Some metabolic aspects of the obese-hyperglycemic syndrome in mice. Diabetologia <u>3</u>, 222, 1966.

41. Coleman, D.L. and Hummel, K.P.: The influence of genetic background on the expression of the obese (ob) gene in the mouse. Diabetologia <u>9</u>, 287, 1973.

42. Herbai, G., Westman, S. and Hellerström, C.: The growth hormone dependent incorporation of sulphate into the costal cartilage of obese-hyperglycemic mice of different ages. Acta Endocrinol. <u>64</u>, 415, 1970.

43. Herbai, G., Westman, S. and Hellerström, C.: Growth hormone as a possible aetiological factor for the obese-hyperglycemic syndrome in mice. Diabetologia <u>6</u>, 48, 1970.

44. Hellerström, C., Westman, S., Herbai, G., Petersson, B., Westman, J., Borglund, E. and Ostensson, C.G.: Pathogenic aspects of the obese-hyperglycemic syndrome in mice (genotype obob): II. Extrapancreatic factors. Diabetologia <u>6</u>, 284, 1970.

45. Wykes, A.A., Christian, J.E. and Andrews, F,N.: Radioiodine concentration and thyroid weight in normal, obese and dwarf strains of mice. Endocrinol. <u>62</u>, 535, 1958.

46. Goldberg, R.C. and Mayer, J.: Normal iodine uptake and anoxia resistance accompanying apparent hypometabolism in hereditary obese-hyperglycemic syndrome. Proc. Soc. Exptl. Biol. Med. <u>81</u>, 323, 1952.

47. Koschinsky, Th., Gries, F.A. und Herberg, L.: Regulation der Glycerokinase durch Insulin in Fettzellen und Leber von genetisch fettsüchtigen Mäusen (BH-ob). Hoppe Seyler's Z. Fys. Chem. 351, 1312, 1970.

48. Koschinsky, Th., Gries, F.A. and Herberg, L.: Glycerol kinase activity in isolated fat cells of BH-ob mice. Horm. Metao. Res. 2, 185, 1970.

49. Koschinsky, Th., Gries, F.A. and Herberg,L.: Regulation of glycerolkinase by insulin in isolated fat cells and liver of Bar Harbor Obese Mice. Diabetologia 7, 316, 1971.

50. Salmon, W.D., Du Vall, M.R., Thompson, E.Y.: Stimulation by insulin in vitro of incorporation of (^{35}S) sulphate and (^{14}C) leucine into protein-polysacharide complexes, (^{3}H) uridine into RNA, and (^{3}H) thymidine into DNA of costal cartilage from hypophysectomized rats. Endocrinol. 82, 493, 1968.

51. Stauffacher, W., Crofford, O.B., Jeanrenaud, B., and Renold, A.E.: Comparative studies of muscle and adipose tissue metabolism in lean and obese mice. Ann. N.Y. Acad. Sci. 131, 528, 1965.

52. Batt, R. and Mialhe, P.: Insulin resistance of the inherently obese mouse obob. Nature 212, 289, 1966.

53. Genuth, S.M.: Hyperinsulinism in mice with genetically determined obesity. Endocrinol. 84, 386, 1969.

54. Lernmark, A. and Hellman, B.: The β-cell capacity for insulin secretion in microdissected pancreatic islets from obese-hyperglycemic mice. Life Sciences 8, 53, 1969.

55. Idahl, L.A. and Martin, J.M.: Stimulation of insulin release by a ventro-lateral hypothalamic factor. J. Endocrinol. 51, 601, 1971.

56. Martin, J.M., Mok, C.C., Penfold, J., Howard, N.J. and Crowne, D.: Hypothalamic stimulation of insulin release. J. Endocrinol. 58, 681, 1973.

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STELLINGEN

I

Om de kans op het optreden van een tweede "thalidomide-drama" zo gering mogelijk te maken, zal de overheid op zeer korte termijn moeten komen tot het instellen van een algehele registratie van congenitale afwijkingen Tot dit doel dienen artsen wettelijk te worden verplicht door hen geconstateerde aangeboren afwijkingen te melden aan een centrale instantie

II

De hypothese van Renwick, inhoudende dat anencephalie en spina bifida bij pasgeboren kinderen meestal veroorzaakt worden doordat de moeder tijdens de zwangerschap een onbekende stof, aanwezig in bepaalde aard appelen, consumeert, is waarschijnlijk slechts een gedeeltelijke verklaring voor het optreden van deze aangeboren misvormingen

<div align="right">Renwick, Br J Prev Soc Med <u>26</u>, 67-88 (1972)</div>

III

Door het in toenemende mate wegvallen van de selectiedruk tegen erfelijke ziekten zal de kwaliteit van de mens als genetische soort verminderen, hetgeen een gevaar inhoudt voor zijn voortbestaan

IV

Het tot stand komen van absolute koppeling van genen d m v inversies is een niet voor alle soorten bruikbaar principe voor het bij elkaar houden van gunstige genencombinaties

V

De progressiviteit in de tarieven van de inkomstenbelasting gecombineerd met de aftrekbaarheid van de rente op geldleningen, dragen op een onrechtvaardige manier bij tot het vergroten van verschillen in financiële welstand.

VI

Gezien de plaatselijk zeer hoge bevolkingsdichtheid in Nederland lijkt het niet zinvol om het honorarium van zittende burgemeesters te relateren aan het aantal inwoners van de gemeente.

Nijmegen, 25 april 1974   H.F.P. JOOSTEN