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

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 "hypoithroidism" read "hypothyroidism".

page 59, 2nd line from the top:
for "Dissretation" 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-immunological" read "tissue-immunological".
PATHOGENESIS OF THE OBESE-HYPERGLYCEMIC

SYNDROME IN MICE (GENOTYPE $^{ob}_{ob}$)
Promotor:
Prof. Dr. H. D. Berendes

Co-referent:
Dr. P. H. W. van der Kroon
PATHOGENESIS OF THE OBESE-HYPERGLYCEMIC SYNDROME IN MICE (GENOTYPE $^{ob}_{ob}$)

PROEFSCHRIFT
TER VERKRIJGING VAN DE GRAAD VAN DOCTOR IN DE WISKUNDE EN NATUURWETENSCHAPPEN AAN DE KATHOLIEKE 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

1974

DRUK STICHTING STUDENTENPERS NIJMEGEN
aan vader en moeder

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

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

The ultimate aim of this study was the definition of the primary genetic defect in homozygous obese mice by performing a thorough investigation of the pathogenesis of the obese-hyperglycemic 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 positive 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 forty years of age. Diabetics have a higher risk for some diseases such as, nephropathy, neuropathy, retinopathy and vascular diseases (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 metabolic anomalies is commonly found to be associated with both diseases. Examples such as a certain hyperinsulinemia and abnormalities 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 carbohydrates, several drugs can be used which either improve the island function or insulin sensitivity. In severe cases, exogenous 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 virtually impossible to prevent the development of diabetes and obesity, in particular if these diseases are determined genetically. This situation will persist as long as the etiology of
these diseases is not completely understood. Considering the high incidence of obesity and diabetes in man, and its economical, psychological and sociological implications, it seemed appropriate to perform some fundamental studies on the etiology of these diseases as a contribution to a better understanding of the primary causes in particular in genetically determined 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 laboratory 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.

<table>
<thead>
<tr>
<th>Dominance</th>
<th>Gene Symbol</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>monogenic</td>
<td>A&lt;sup&gt;y&lt;/sup&gt;</td>
<td>yellow (9)</td>
</tr>
<tr>
<td></td>
<td>A&lt;sup&gt;vy&lt;/sup&gt;</td>
<td>viable yellow</td>
</tr>
<tr>
<td></td>
<td>A&lt;sup&gt;ly&lt;/sup&gt;</td>
<td>intermediate yellow</td>
</tr>
<tr>
<td>recessive</td>
<td>ob</td>
<td>obese (10)</td>
</tr>
<tr>
<td></td>
<td>ad</td>
<td>adipose (11)</td>
</tr>
<tr>
<td></td>
<td>db</td>
<td>diabetes (12)</td>
</tr>
<tr>
<td>polygenic</td>
<td>NZO</td>
<td>New Zealand Obese (13)</td>
</tr>
<tr>
<td></td>
<td>KK</td>
<td>Japanese obese (14)</td>
</tr>
<tr>
<td></td>
<td>C3Hf x I, F&lt;sub&gt;1&lt;/sub&gt; Wellesley mouse (15)</td>
<td></td>
</tr>
</tbody>
</table>

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 (CTG)
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 disease 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. Destruction 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 (\(\text{ob}^{\text{ob}}\)) has been studied most thoroughly (for review: 6, 16).
2. A brief historical review of studies on the obese-hyperglycemic 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 designated 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 hypercholesteremia (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 adipose 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 adipose 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, increasing serum insulin and blood glucose concentration.
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 hyperinsulinemia (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
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 $^{ob}$. 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 $^{ob}$ $^{ob}$ is to mate mice heterozygous for $^{ob}$. Furthermore, it is only after 4 weeks that the presence of obesity becomes obvious. Before the age of 4 weeks, the external phenotype of the obese mouse is not different from the phenotype 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 $^{ob}$ $^{ob}$ 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 offspring of the mating $^{ob}$ $^{ob}$ $^{ob}$ $^{ob}$, which was used throughout this investigation, is theoretically composed of 25% $^{ob}$ $^{ob}$ animals (obese phenotype) and 75% $^{ob}$ $^{ob}$ and $^{ob}$ $^{ob}$ 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 identification of their genotype at about 4 weeks of age. This simple requirement inhibits the performance of certain experiments and makes other experiments more difficult. Histological and biochemical studies on brain tissue for example, are impossible to carry out with mice younger than 4 weeks because the operation is lethal. Other operations, such as removal of a piece of pancreas tissue to study structural changes of the islands of Langerhans can be successfully performed on very young mice but post-operative events, failure of acceptance by the mother or removal of the suture 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 ele­
ment for an answer to the question what the causal relation
between obesity and hyperinsulinemia is. Is obesity a conse­
quence of, or does it precede 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 ex­
cluded on the basis of the data available. After the establish­
ment of hypothyroidism in the adult obese mouse, an attempt
was made to determine at what time in development hypothy­
roidism becomes manifest.

The studies on thyroid function led to the third aim, an in­
vestigation of the development of reflexes and behavioral
components, of locomotor activity and body growth and a com­
parison 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).
Chapter II.

The enlargement of epididymal adipocytes in relation to hyperinsulinemia in obese-hyperglycemic mice (ob/ob).
Summary

The diameters of epididymal fat cells of 12 to 17 days old obese and normal littermates were compared following operative 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 allele 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, hyperglycemia, 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 epididymal fat body is already clearly enlarged at day 16, however (3).

With respect to the other features it is known that hyperinsulinemia is also detectable at an age of 4 weeks (4, 5), whereas 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 hyperglycemia 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 definition of whether or not obesity does develop before or concomitant with the occurrence of hyperinsulinemia.
Material and methods

Experimental animals
The animals used in this study were male obese mice (genotype ob/ob) and normal mice (ob/+ and +/+ 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 ob/ob, ob/+ and +/+ 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 °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 (OH and +) 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.

<table>
<thead>
<tr>
<th>litter nr.</th>
<th>litter size</th>
<th>mean of median values of epididymal fat cell diameters of normal mice in a litter (μ)</th>
</tr>
</thead>
<tbody>
<tr>
<td>18</td>
<td>6</td>
<td>30</td>
</tr>
<tr>
<td>19</td>
<td>9</td>
<td>25</td>
</tr>
<tr>
<td>20</td>
<td>11</td>
<td>18</td>
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<td>21</td>
<td>9</td>
<td>18</td>
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<td>22</td>
<td>12</td>
<td>17</td>
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<tr>
<td>23</td>
<td>10</td>
<td>25</td>
</tr>
<tr>
<td>24</td>
<td>12</td>
<td>14</td>
</tr>
</tbody>
</table>

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)

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

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.
Curved 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 index of increase in fat cell diameter in obese and the age deviates 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 animals having large cells. Fig. 2 presents an example of a litter with 3 male mice, one $\text{+}$, one $\text{ob}$ and one $\text{ob}$ mouse. At an age of 6 weeks animals of group 3 turned out to be $\text{ob}$ 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 $3 \text{ob} +$ and $3 \text{+}$ by chance is 0.01. This evidently shows that it is possible to differentiate between homozygous $\text{+}$ and heterozygous $\text{ob}$ 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 $+/+$ (median value 19 μ), $ob/+$ (median value 24 μ) and $ob/ob$ (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 $ob$

<table>
<thead>
<tr>
<th>mouse nr.</th>
<th>fat cell diameter</th>
<th>genotype predicted on the basis of fat cell diameters</th>
<th>obese in the first litter</th>
<th>obese in the second litter</th>
</tr>
</thead>
<tbody>
<tr>
<td>66</td>
<td>interm.</td>
<td>$ob/+$</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>67</td>
<td>small</td>
<td>$+/+$</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>68</td>
<td>interm.</td>
<td>$ob/+$</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>69</td>
<td>small</td>
<td>$+/+$</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>70</td>
<td>small</td>
<td>$+/+$</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>71</td>
<td>interm.</td>
<td>$ob/ob$</td>
<td>+</td>
<td></td>
</tr>
</tbody>
</table>
**Blood glucose**

Fig. 3, which presents the results of colorimetric determinations 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).

![Blood glucose concentration of obese and non-obese mice in relation to age.](image)

From 8 weeks on the difference between obese and non-obese animals 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 insulin, 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, $0.001 < P < 0.01$).

![Graph showing plasma immunoreactive insulin concentration 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.]

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.
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 concentration 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.
Discussion

Obese mice with established obesity have greatly enlarged fat cells in their epididymal fat body (8). Hyperplasia of epididymal adipocytes contributes little to the increased weight of this fat pad (9). Five months old obese mice have cell diameters of around 95 μ, normal mice around 71 μ (8). (Volumes of $44.8 \times 10^4$ and $18.7 \times 10^4 \mu^3$ respectively). At day 14 these figures are 27 μ and 21 μ (volumes $1 \times 10^4$ and $0.5 \times 10^4 \mu^3$) 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 intermediate cell size, it seems that the ob allele is incompletely dominant, in other words that the ob allele is expressed in the heterozygotes. A similar conclusion may be derived from the data on the oxidation of glucose in vitro by the epididymal fat body of 3-5 months old mice homozygous or heterozygous 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 accumulation 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). However, it could be argued that the quantity of free fatty acids, used as a parameter in these experiments, does not give adequate 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 reduced 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 manifest only after 4 weeks (see also: Genuth et al., 1971 (5)), whereas the increase in fat cell diameter begins already after 13 days. An explanation can be found in the deposition 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 the 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 development.

Acknowledgement
The authors wish to thank Prof. H.D. Berendes for the fruitful discussions, Mr. A.J.M. Buis and Mr. A.J.M. Coenen for breeding and maintaining the mice and Mrs. C.M.P. Sykora for her contribution.
References


The role of the thyroid in the development of the obese-hyperglycemic syndrome in mice (\textsuperscript{ob/ob}).
Summary

Some aspects of thyroid activity in obese mice were investigated. Protein Bound Iodine (PBI), Hormonal Iodine (HI), Total Thyroxin (TT₄) 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 genetically obese mouse is hypothyroid. This conclusion is supported 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 hypothyroidism 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 indicative 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 131I 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 postnatal 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 essential 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 \( \text{ob}^\text{ob} \)) and non-obese littermates (\( \text{ob}^+ \) and \( +^+ \)) 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 environment and propagated by mating heterozygous parents. The animals were fed an All Hope Farms diet RMH-B containing 0.40 \( \mu \)g iodine per gram. For the experiments with equalized exogenous iodine supply, mice were fed a iodine-poor diet with 0.04 \( \mu \)g iodine per gram for 14 days before the experiment 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°C. The temperature dependent voltage difference between the two couples was registered by a voltmeter in \( \mu \)V, the voltage difference being 18.4 \( \mu \)V per 1°C.

Thyroxin injections
Thyroxin (Fluka) was injected intraperitoneally. The solution was made 0.15 M NaCl (pH 9.0) containing 2.5 \( \mu \)g 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 O₂-consumption was recorded in µl/min/25 mg fresh liver and as µl/min/mg protein. Protein determinations were done after Lowry et al. (1951). Equal samples of homogenate without substrate were used as controls.

**Uptake and release of radioiodine from the thyroid**

Mice were injected with 5-7 µCi carrier-free Na¹³¹I (spec. act. 6-20 mCi/µg I, Radiochemical Centre, Amersham). Thyroidal radioiodine content was determined by in vivo counting 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). ¹³¹I 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 radioiodine 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 successively with 8.0 ml 0.1 N HCl, 8 ml 1.0 N 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 H₂SO₄ 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 μm 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 μm is read. On semilog paper the relation between concentration on the decimal axis and absorbance on the logarithmic axis is linear.

**Determination of Protein Bound Iodine (PBI)**

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

**Determination of total Thyroxin TT₄**

The TT₄ 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). Determinations 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):

\[ \text{volume} = \text{constant} \times b \cdot l^2 \]

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):

\[ \text{apical cell width} = \text{constant} \times d \cdot \frac{1}{n} \]

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 temperature between the minimum in the light period and the maximum in the dark period is 2.0°C in obese and 1.5°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

<table>
<thead>
<tr>
<th>age (weeks)</th>
<th>non-obese ♀♀</th>
<th>non-obese ♂♂</th>
<th>obese ♀♀</th>
<th>obese ♂♂</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>37.99 ± 0.09 (12)</td>
<td>37.43 ± 0.23 (14)</td>
<td>36.70 ± 0.06 (10)</td>
<td>36.64 ± 0.07 (11)</td>
</tr>
<tr>
<td>8-9</td>
<td>37.80 ± 0.08 (5)</td>
<td>37.42 ± 0.10 (5)</td>
<td>36.43 ± 0.10 (5)</td>
<td>36.46 ± 0.07 (5)</td>
</tr>
</tbody>
</table>

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.
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.

![Graph showing body temperature over age for non-obese and obese mice](image)

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 °C to about 1.0 °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

<table>
<thead>
<tr>
<th>age (weeks)</th>
<th>obese</th>
<th>non-obese</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>36.64 ± 0.07 (11)</td>
<td>37.43 ± 0.09 (12)</td>
</tr>
<tr>
<td>5-6</td>
<td>36.09 ± 0.12 (9)</td>
<td>36.89 ± 0.18 (10)</td>
</tr>
<tr>
<td>8-9</td>
<td>36.46 ± 0.07 (5)</td>
<td>37.42 ± 0.10 (5)</td>
</tr>
<tr>
<td>9-10</td>
<td>36.34 ± 0.08 (5)</td>
<td>37.32 ± 0.10 (12)</td>
</tr>
<tr>
<td>20</td>
<td>36.28 ± 0.10 (11)</td>
<td>37.70 ± 0.07 (11)</td>
</tr>
</tbody>
</table>

GTG obese  non-obese controls
20  37.68 ± 0.09 (10)  37.60 ± 0.10 (10)

Values are mean ± 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.
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 °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 TT4 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 TT4 in obese mice of 5 months, a similar decrease has been detected.
Table 3

Plasma concentrations of HI, PBI and TT4 and 131I uptake and release of the thyroid in obese and non-obese mice at various ages

<table>
<thead>
<tr>
<th>Age (weeks)</th>
<th>Animals</th>
<th>HI (μg/100 ml)</th>
<th>PBI (μg/100 ml)</th>
<th>TT4 (μg/100 ml)</th>
<th>131I uptake</th>
<th>t1/2 thyroidal 131I</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>obese</td>
<td>0.55 ± 0.10 (9)**</td>
<td></td>
<td></td>
<td>9.86 ± 1.47 (9)**</td>
<td>3.33 ± 0.34 (9)**</td>
</tr>
<tr>
<td></td>
<td>non-obese</td>
<td>0.92 ± 0.10 (6)</td>
<td></td>
<td></td>
<td>18.79 ± 1.80 (9)</td>
<td>2.09 ± 0.31 (9)</td>
</tr>
<tr>
<td>9-11</td>
<td>obese</td>
<td>0.39 ± 0.10 (7)**</td>
<td></td>
<td>1.43 ± 0.45 (4)</td>
<td>7.40 ± 0.37 (11)**</td>
<td>2.79 ± 0.21 (11)**</td>
</tr>
<tr>
<td></td>
<td>non-obese</td>
<td>0.66 ± 0.07 (11)</td>
<td></td>
<td>1.83 ± 0.32 (4)</td>
<td>15.34 ± 2.08 (11)</td>
<td>4.13 ± 0.35 (11)</td>
</tr>
<tr>
<td>20-24</td>
<td>obese</td>
<td>0.98 ± 0.06 (5)</td>
<td>1.60 ± 0.11 (7)**</td>
<td>1.28 ± 0.14 (4)**</td>
<td>6.81 ± 0.64 (15)**</td>
<td>3.96 ± 0.47 (10)</td>
</tr>
<tr>
<td></td>
<td>non-obese</td>
<td>1.37 ± 0.11 (5)**</td>
<td>2.31 ± 0.17 (6)</td>
<td>1.86 ± 0.10 (5)</td>
<td>15.27 ± 0.74 (15)</td>
<td>3.95 ± 0.55 (10)</td>
</tr>
</tbody>
</table>

Values are mean ± SEM. Number of observations in parentheses.

HI and PBI are expressed as μg I/100 ml plasma and TT4 as μg thyroxin/100 ml plasma.

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

As a parameter for turnover of thyroidal radiiodine, 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 radiiodine incorporated by the non-obese thyroid. The release of radiiodine 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).

![Graph showing $^{131}$I uptake by the thyroid in obese and non-obese mice.](image)

**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 on 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 radiiodine were started at 48 hours after injection of 5–7 μCi of the radioactive isotope. At the subsequent days radiiodine content was determined and expressed as a percentage of the 48 hour count. The mean and the SEM is indicated.
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 the second group (9 obese and 9 non-obese males) was fed an iodine-poor diet and supplemented with a daily injection of 2 $\mu$g 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 $\mu$g per day.

Under standardized dietary conditions (low iodine), obese and non-obese mice were supplied with 2 $\mu$g 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.

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

Values are mean ± 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 expressed as the biological half-life of thyroidal radiiodine 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 obese 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 °C and in non-obese mice 0.8 °C. Under these conditions obese mice can attain the body temperature of non-injected non-obese mice.

![Graph showing body temperature in obese and non-obese mice](image)

_fig. 7._ The influence of thyroxin injections on body temperature 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 μg thyroxin/100 g/day. The response of the two obese groups was similar. Therefore, the observations were pooled. The mean and the SEM is indicated.
Induction of $\alpha$-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.

<table>
<thead>
<tr>
<th>animals</th>
<th>thyroxin dose</th>
<th>GPDH act. before injection</th>
<th>after 4 days injections</th>
<th>increase</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>per 25 mg liver</td>
<td>per mg protein</td>
<td>per 25 mg liver</td>
</tr>
<tr>
<td>non-obese</td>
<td>6</td>
<td>1.61 ± 0.13</td>
<td>0.48 ± 0.05</td>
<td>2.19 ± 0.08</td>
</tr>
<tr>
<td>obese</td>
<td>3</td>
<td>1.84 ± 0.10</td>
<td>0.26 ± 0.03</td>
<td>3.42 ± 0.13</td>
</tr>
<tr>
<td>obese</td>
<td>6</td>
<td>3.16 ± 0.11</td>
<td>0.44 ± 0.03</td>
<td>3.16 ± 0.11</td>
</tr>
</tbody>
</table>

GPDH activity is expressed as μL O₂/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.
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 influence 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 picture (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 worthwhile 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 expected 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 endogenous specific activity of $^{131}\text{I}$ 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 $^{131}\text{I}$ 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 $^{131}\text{I}$ uptake between obese and non-obese mice receiving a normal diet is caused by the difference in $^{127}\text{I}$ 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 individual 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 metabolic 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 °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-glycerophosphate dehydrogenase (fig. 7 and table 5). The expected hypersensitivity was confirmed: body temperature rose 1.6 °C in obese and 0.8 °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 body temperature of these young obese mice could reflect a low BMR as compared to the non-obese mice. The results on O2 con-
consumption of obese mice of 14-28 days as reported by Fried et al. (28) can be interpreted similarly. Both parameters, body temperature and O₂ consumption could be consequences of a decreased BMR resulting from hypothyroidism.

Hypothyroidism may be the explanation for various aspects of the obese-hyperglycemic syndrome. It has a calory saving effect resulting 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 decreased 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 probably results from a decreased number of cells. Since the thyroid hormone is considered to be a hormone affecting postnatal development of the central nervous system, the defective development of the brain in obese mice can very well be a result of congenital hypothyroidism.

Acknowledgements

The authors thank Prof. H.D. Berendes, Dr. P.W. Kloppenburg and Dr. T.J. Benraad (Dept. Int. Med.) for the fruitful discussions. Thanks are due to Drs. H.A. Ross and Dr. A.P. Jansen (Dept. Int. Med.) for performing the assays of T₄ and PBI, to Mr. A.J.M. Buis and Mr. A.J.M. Coenen for breeding and maintaining the mice and Mrs. W.M. Kortekaas for her valuable contribution.
References


Chapter IV.

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

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 characterized 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 conclusion that obese mice are hypothyroid.

Hypothyroidism could account for a number of characteristics of the syndrome: low basal metabolic rate, low body temperature, 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 activity 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 development 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 reflexes 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 obtained 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 marked individually and checked for obesity at 4-5 weeks.

Locomotory activity and reflexes
Because the activity of young mice is significantly influenced 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 observations 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 averages over 3 days.

Body weight
Each of a group of 100 mice was weighed every day from birth until the 16th day. The data obtained from 9 animals 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 percentage 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 $p < 0.05$). 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.
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 logarithmically.
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 differences 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 significance 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.
Table 1.

Intake of solid food

<table>
<thead>
<tr>
<th></th>
<th>5 weeks</th>
<th>5 months</th>
</tr>
</thead>
<tbody>
<tr>
<td>obese</td>
<td>$6.2 \pm 0.7$ (6)*</td>
<td>$6.4 \pm 0.9$ (14)*</td>
</tr>
<tr>
<td>non-obese</td>
<td>$4.3 \pm 0.3$ (6)</td>
<td>$5.5 \pm 0.3$ (16)</td>
</tr>
<tr>
<td>hyperphagia</td>
<td>44%</td>
<td>16%</td>
</tr>
</tbody>
</table>

efficiency of weight gain at 5 weeks; obese $18.8 \pm 2.5$, non-obese $12.7 \pm 0.8$

intake expressed as gr/day
the mean and the SEM are given with the number of observations in parentheses

efficiency is defined as \[
\frac{\text{weight increase per day}}{\text{food intake per day}} \times 100\%
\]
determined during a 3 day period

*significant at $P < 0.01$ (Student t-test)
Fig. 3. The relative weight gain:
\[
\frac{(\text{weight on day } n+1 - \text{weight on day } n)}{\text{weight on day } n} \times 100\%
\]
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.
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 conclusion; inactivity being a consequence rather than a cause of obesity. Their conclusion is based on the fact that decreased 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 postnatal week, caloric expense can hardly differ between obese and non-obese mice because the early postnatal mouse is poikilothermic. The variation in metabolic rate with environmental temperature 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 homiothermia 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.

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References

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

By mating mice heterozygous for the recessive gene obese $\frac{ob}{ob}$ with mice homozygous for the recessive gene dwarf $\frac{+}{+} \frac{dw}{+}$ 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 $\frac{ob}{ob}$ 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°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).
Breeding scheme for the production of obese dwarf mice

The $\frac{+}{-}$ animals are normally sterile, but males of this type were made fertile by daily subcutaneous transplantation of a pituitary from a normal male mouse. The $\frac{ob}{+}$ animals of generation II were identified by back-crossing to the parental genotype $\frac{ob}{+}$. 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 heterozygotes in a ratio of 1:1:2 (generation II, fig. 1). Since the obese gene is recessive, heterozygous animals are phenotypically 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 maintained by mating heterozygous dwarf females with fertile homozygous dwarf males. These matings result in an offspring consisting 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 $ob +$ were mated with males homozygous for dwarf $dw$. 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 capillaries. Blood samples were diluted three fold with a buffered saline-albumin solution and centrifuged in microtubes. 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 densitometrically on a Zeiss densitometer.
Results

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 offspring, 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 animals, 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 aforementioned 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).
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.

Table 1.

Segregation data of matings \( \frac{\text{ob} \ x \ \text{ob}}{+ \ +} \times \frac{\text{dw}}{+ \ +} \)

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>dwarf</th>
<th>non-dwarf</th>
<th>total</th>
</tr>
</thead>
<tbody>
<tr>
<td>obese</td>
<td>20 / 24 (26)</td>
<td>72 (78)</td>
<td>92 / 96 (104)</td>
</tr>
<tr>
<td>non-obese</td>
<td>81 / 77 (78)</td>
<td>241 (233)</td>
<td>322 / 318 (311)</td>
</tr>
<tr>
<td>total</td>
<td>101 (104)</td>
<td>313 (311)</td>
<td>414</td>
</tr>
</tbody>
</table>
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 difference in loading of the gels since the equivalent number of pituitaries used for GH determination in dwarf homogenates is 11 times higher than in the non-dwarf homogenates.

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 compared 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, although 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**

<table>
<thead>
<tr>
<th></th>
<th>nor-obese dwarf</th>
<th>nor-dwarf</th>
<th>obese non-dwarf</th>
<th>non-obese dwarf</th>
<th>obese dwarf</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>body weight (gr)</strong></td>
<td>21.8 ± 0.5 (10)</td>
<td>24.4 ± 0.8 (10)</td>
<td>37.4 ± 1.2 (7)</td>
<td>38.3 ± 1.3 (8)</td>
<td>7.2 ± 0.4 (8)</td>
</tr>
<tr>
<td><strong>plasma insulin (μJ/ml)</strong></td>
<td>26.1 ± 7.9 (3)</td>
<td>25.9 ± 6.8 (8)</td>
<td>326.9 ± 46.6 (7)</td>
<td>232.0 ± 44.9 (8)</td>
<td>14.5 ± 3.6 (8)</td>
</tr>
<tr>
<td><strong>blood glucose (mg%)</strong></td>
<td>96.2 ± 4.5 (10)</td>
<td>91.8 ± 3.9 (9)</td>
<td>114.4 ± 5.7 (7)</td>
<td>196.8 ± 29.5 (8)</td>
<td>66.4 ± 7.0 (7)</td>
</tr>
</tbody>
</table>

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.
Adiposity of the obese dwarf mouse

Obese dwarf on the left and non-obese dwarf 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 considered 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 incorporation 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 level.

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 combined. 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 \( \text{ob} \) and dwarfism \( \text{dw} \), 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 homogenates of non-dwarf mice is identical with GH. This band is absent in the electropherograms from non-obese dwarf and obese dwarf mice, indicating that these mice do not have significant 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 overweight 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 the 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 \( \text{ob} \over \text{dw} \), the characteristic syndrome of the obese hyperglycemic 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 aggravate 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. Because 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

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Chapter VI

Discussion

The results of the present investigation are still insufficient to provide a clear answer to the question as to what should be considered the primary defect of the obese-hyperglycemic 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 available data, including the sequence in which the various features of the obese-hyperglycemic syndrome become manifest during 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 distinguished from the 14th day of development, it seems likely that a difference in either lipogenesis and/or lipolysis between 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 diabetes, is the primary defect or a direct consequence thereof. It can be excluded that obesity is a consequence of hyperinsulinemia on account of the plasma insulin data and on the basis of submicroscopic studies of the islands of Langerhans. 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 genetically obese mouse could be explained on the basis of a hypothyroidism. 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 hypothyroidism may occur as early as the 10th day of postnatal development. Moreover, the latest 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 development. Also these features may be seen as a consequence of hypothyroidism 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 muscular activity, the high growth rate is supported by a congenitally higher caloric intake; i.e. the obese mouse could be congenitally hyperphagic. 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 dissertation suggest that the obese-hyperglycemic syndrome in mice originates from a congenital hypothyroidism and a congenital 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-hyperglycemic syndrome develops in the $\text{ob/ob}$ 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. obtained 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, Herbal, 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
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 normalization of body weight (52). Because there are strong indications 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 obvious that muscle insulin insensitivity could hardly be considered 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 hypothesis 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 hypersensitivity 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, hyperinsulinemia and insulin resistance are secondary events. Our results on the growth of the epididymal adipose tissue supports this concept. Ideas about the mechanism bringing about 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 opinion, 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 significance 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 secretion kinetics in vitro, they were not viable after transplantation into the acceptor animals. Other laboratories were also unsuccessful in their efforts (Bray, personal communication). A possible explanation for the failure to confirm the results of Strautz may be found in differences in immunological characteristics between the islands of the donor mice and the host, causing rapid degeneration of the implanted 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 could still be present in the genetic background responsible for the tissue-immunological 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 possibilities 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 likely 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 insulin 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 cannot 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 \( \text{ob/ob} \) is a complicated 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 syndrome.

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 hyperphagia 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.
Het obesitas-diabetes syndroom in de muis $^{ob}_{ob}$ is een ingewikkeld syndroom, dat in zijn volwaardige vorm gekenmerkt wordt door endocrine-, metabole- en gedragsafwijkingen. In een poging tot identificatie en lokalisaatie van het primaire genetische defect werden een aantal eigenschappen van het syndroom zoals: hypothyroïdie, hyperglycémie, hyperinsulinemie, verlaagde locomotorische activiteit, 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 vanaf 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 net congenitaal aanwezig zijn van deze hypothyroidie.

De uit dit onderzoek verkregen gegevens leiden tot de conclusie dat noch een afwijkende groeihoormoon produktie, noch de hyperinsulinemie of de insuline resistentie beschouwd kunnen worden als het primaire genetische defect in de etiologie 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 hypothalamus.
References


Curriculum vitae


Vanaf april 1969 tot juni 1973 was hij werkzaam, als wetenschappelijk medewerker, op de afdeling Genetica aan de Universiteit te Nijmegen, aanvankelijk met als hoofd Prof. Dr. S.J. Geerts en later Prof. Dr. H.D. Berendes. Vanaf 1 juli 1973 is hij research medewerker binnen de sectie Reproductie Toxicologie van de afdeling Drug Safety van Organon B.V. te Oss.
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 pasgeborenen 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. 


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 dmv inversies is een niet voor alle soorten bruikbaar principe voor het bij elkaar houden van gunstige genencombinaties.
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