Effect of Weight and Cell Size on Hormone-Induced Lipolysis in New Zealand Obese Mice and American Obese Hyperglycemic Mice*

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Summary. In NZO mice fat cell size is strongly correlated to increasing body weight; FFA and glycerol release from adipose tissue under basal conditions and after stimulation with norepinephrine, ACTH, DB-cyclic AMP and theophylline increase with body weight and fat cell size. Normal weight NZO mice and lean littermates of *obob* mice respond similarly to lipolytic agents. — In *obob* mice cell size and body weight are correlated until a body weight of about 60 g is reached. Older *obob* mice show a decrease in cell size with increasing body weight. There is no correlation between stimulated lipolysis and fat cell size. In these animals an inverse relationship between serum insulin and stimulated lipolysis in adipose tissue was observed. — It is concluded that NZO mice exhibit a type of hypertrophic obesity and *obob* mice a type of hyperplastic obesity, which may be differentiated by fat cell morphology and the sensitivity of adipose tissue to lipolytic agents.

Effet du poids et du volume des cellules adipeuses sur la lipolyse après stimulation hormonale des souris NZO et obob

Résumé. Chez la souris NZO, il existe une étroite corrélation entre la dimension de la cellule adipeuse et l'augmentation du poids corporel. Chez ces animaux, la libération des AGL et du glycérol à partir du tissu adipeux augmente en fonction du poids et de la dimension des cellules adipeuses, que ce soit dans les conditions de base ou après stimulation par la noradrénaline, l'ACTH, le DB-AMP cyclique et la théophylline. La réponse aux agents lipolytiques est la même chez la souris NZO normopondérale et les souris normales provenant de la même niche que les souris *obob*. Chez ces dernières, la dimension de la cellule adipeuse et le poids corporel sont en corrélation jusqu'à un poids corporel d'environ 60 g. Chez des souris *obob* plus âgées, une diminution de la dimension de la cellule est observée lorsque le poids augmente. Aucune corrélation ne peut être établie entre la stimulation de la lipolyse et la dimension de la cellule adipeuse. Il existe une corrélation inverse entre l'insulinémie et la stimulation de lipolyse dans le tissu adipeux. — Il est

conclu de ces expériences que les souris NZO pourraient être atteintes d'un type d'obésité hypertrophique et les souris *obob* d'un type d'obésité hyperplasique, dont la distinction semble pouvoir se faire par l'examen morphologique de la cellule adipeuse et par la sensibilité du tissu adipeux aux agents lipolytiques.

Einfluß von Körpergewicht und Zellgröße auf die hormoninduzierte Lipolyse des epididymalen Fettgewebes bei NZO- und obob-Mäusen

Zusammenfassung. Bei NZO-Mäusen besteht eine enge Korrelation zwischen Zunahme des Körpergewichtes und Vergrößerung der Einzelfettzelle. Unter Basalbedingungen sowie nach Stimulierung mit Noradrenalin, ACTH, DB-cyclo AMP und Theophyllin nimmt die Freisetzung von Glycerin und freien Fettsäuren aus dem Fettgewebe bei NZO-Mäusen mit steigendem Körpergewicht und Vergrößerung der Fettzelle zu. — Junge, nicht fettstüchtige NZO-Mäuse und nicht fettstüchtige Wurfgeschwister hyperglykämischer *obob*-Mäuse reagieren in ähnlicher Weise auf lipolytische Stimuli. — Bei *obob*-Mäusen besteht zwischen der Fettzellgröße und dem Körpergewicht eine Korrelation, bis die Tiere ein Gewicht von ungefähr 60 g erreicht haben. Bei älteren *obob*-Mäusen tritt trotz einer weiteren Zunahme des Körpergewichtes eine Abnahme der Fettzellgröße auf. Es besteht keine Korrelation zwischen stimulierter Lipolyse und Fettzellgröße. Die Höhe des Seruminsulinspiegels und die stimulierte Lipolyse des Fettgewebes verhalten sich bei diesen Tieren umgekehrt proportional. — Es wird geschlossen, daß NZO-Mäuse den Typ einer hypertrophischen Fettsucht und *obob*-Mäuse den Typ einer hyperplastischen Fettsucht verkörpern, die sich hinsichtlich der Fettzellmorphologie und der Empfindlichkeit des Fettgewebes auf lipolytische Agenzien voneinander unterscheiden.

Key-words: Spontaneous diabetes, mutation *obob*, NZO mice, diabetes in mice, obesity in mice, hereditary obesity, adipose tissue, lipolysis, lipogenesis, size of fat cells.

Obesity in New Zealand obese mice (NZO) and American obese hyperglycemic mice (*obob* mice) seems to be due both to increased lipogenesis and decreased lipolysis. Increased lipogenesis has been demonstrated in *obob* mice by an increased conversion of labelled carbohydrates into fatty acids of the liver and carcass [13]. In NZO indirect evidence was derived from decreased oxidation of glucose to CO₂ as compared with normal controls [22]. Decreased release of free fatty acids from adipose tissue was found by Marshall and Engel [16] who concluded that *obob* mice during fasting exhibit decreased fat mobilization. However, the

release of free fatty acids from adipose tissue of starved NZO mice into the incubation medium is significantly greater than from adipose tissue of fed NZO mice [24].

Differences in adipose tissue lipolysis do not only occur between different strains of mice but also between animals of the same species, of different weight and age. Epinephrine-stimulated lipolysis decreases in *obob* mice with increasing age [16]. The uptake of ¹⁴C-glucose by epididymal fat pads in *obob* mice also declines with increasing age [11]. Since the differences in the lipolytic activity of adipose tissue during fasting in these two strains of mice suggest different types of metabolic obesity, the present study was carried out to evaluate contribution of weight, cell size and serum insulin to fat mobilization in these animals.

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Materials and Methods

The following animals were used: Swiss albino mice (Müller, Haan, Germany) New Zealand obese mice (NZO mice) from the strain described by Bielschowsky, M.-F. Bielschowsky [3]. C 57 BL mice and American obese hyperglycemic mice (*obob* mice) and their lean littermates obtained from the Jackson Memorial Laboratories, Bar Harbor, Maine, USA. They were maintained as previously described [8], allowed free access to food and tap water. Studies were performed with epididymal adipose tissue. Animals were killed by decapitation and bleeding and sufficient quantities of tissue were obtained for both the metabolic studies and the determination of cell size.

Fig. 1. Body weight and fat cell diameter of Swiss Albino Mice I, C57BL mice II, New Zealand obese mice III, American obese-hyperglycemic mice (*obob*) IV. The number of animals is indicated in parentheses mean $\pm$ SEM

Incubation of tissue. 40–60 mg of epididymal adipose tissue were incubated at 37°C in a metabolic shaker oscillating at 80 cycles per minute. Pre-incubation was performed for 30 min in a modified Krebs-Ringer bicarbonate buffer containing 4 g% human albumin (KRBA) and 5.55 mM glucose. Thereafter the fat pads were transferred to glucose-free KRBA and incubated for 2 h. The reactions were terminated by chilling in ice.

Lipolytic agents. The effect of the following agents was studied: Theophylline, 10⁻²M (Merck, Darmstadt, Germany) DB 3,5-cyclic AMP, 10⁻³M (Boehringer, Mannheim, Germany) Norepinephrine 0.58 $\times$ 10⁻³M (Hoechst, Frankfurt, Germany) ACTH, 125 μ U/ml (Ciba AG, Basel, Switzerland).

Analytical procedures. Free fatty acids (FFA) in buffer were determined by a modification [7] of the method of Itaya and Ui [9]. Glycerol was measured

enzymatically according to Kreutz [12] and FFA and glycerol release was related to μ Mol esterified fatty acids (EFS) of the incubated adipose tissue as determined by the method of Antonis and coworkers [1] and calculated for 10⁵ fat cells.

Determination of cell size. Isolated fat cells were liberated from the tissue according to the method of Rodbell [19]. The washed cells were stained with methylene blue, suspended in siliconized counting chambers and measured microscopically.

Calculations. The average lipid content of the single fat cell was calculated according to Faulhaber [4], using the following formula

$$V = \frac{\pi \times d^3}{6}$$

$$G = \pi \times \frac{d^3 \times 0.915}{6 \times 10^6} \mu\text{g triglyceride}$$

$$V = \text{volume}$$

$$d = \text{average fat cell diameter}$$

$$0.915 = \text{density of triolein.}$$

From the lipid content of the single fat cell and the EFA content of the tissue, the cell number was calculated.

Results

Epididymal fat cell diameter and volume in Swiss albino, C 57 BL, obob and NZO mice. When epididymal fat cell diameters of animals of same weight are compared (Fig. 1, Table 1) no difference is observed between Swiss albino mice, NZO mice and C57 BL mice. However, in lean litters of *obob* mice the diameters are about 40% greater. In *obob* mice, cell size and volume increase only till the animals have gained an average weight of 55 g whereafter, in spite of a

Table 1. Body weight (g), diameter (μ) and volume ($\times 1000 \mu^3$) of fat cells in epididymal adipose tissue in four types of mice

mean $\pm$ SEM	The number of animals is indicated in parentheses		
animals	body weight	diameter	volume
swiss albino mice	25–30 (6)	45.7 $\pm$ 1	182 $\pm$ 0.002
C57BL mice	26–30 (6)	45.5 $\pm$ 1	181 $\pm$ 0.002
	20–29* (16)	62.6 $\pm$ 1	469 $\pm$ 0.002
american obese	30–39 (12)	89.8 $\pm$ 2	1381 $\pm$ 0.015
hyperglycemic mice	40–49 (16)	114.1 $\pm$ 2	2837 $\pm$ 0.015
	50–59 (12)	120.7 $\pm$ 3	3358 $\pm$ 0.052
(<i>obob</i>)	60–69 (7)	110.0 $\pm$ 3	2542 $\pm$ 0.052
* = lean	70–79 (13)	102.6 $\pm$ 3	2062 $\pm$ 0.052
littermates			
New Zealand	20–29 (18)	46.1 $\pm$ 1	187 $\pm$ 0.002
obese mice	30–39 (18)	65.4 $\pm$ 4	534 $\pm$ 0.002
	40–49 (10)	79.4 $\pm$ 3	956 $\pm$ 0.052
	60–69 (11)	99.0 $\pm$ 3	1853 $\pm$ 0.052
	> 80 (7)	112.7 $\pm$ 2	2734 $\pm$ 0.015

further increment in weight, the cell diameter decreases. On the other hand, in NZO mice a strong correlation exists between increasing body weight and cell size.

Spontaneous lipolysis in obob and NZO mice. The difference between the two strains of obese hyperglycemic mice becomes more evident when spontaneous lipolysis is compared (Table 2, Fig. 2). Incubation of fat pad specimens results in a free fatty acid release which markedly increases with body weight in both strains. In *obob* mice an increased glycerol release is

Fig. 2. Spontaneous lipolysis with contribution to body weight in American obese-hyperglycemic mice (*obob*) and New Zealand obese mice

Table 2. Spontaneous release of free fatty acids (FFA) and glycerol (glyc) in swiss albino mice = sa, american obese-hyperglycemic mice = obob, New Zealand obese mice with contribution to increasing body weight

animals	body weight (g)	fat cell diameter (μ)	FFA mμmole/10 cells/2 h	glyc mμmole/10 cells/2 h	FFA/glyc
sa	25-30	45.7±1 (6)	35.8±5.6 (6)	18.0±1.6 (6)	2.0
obob	25-33*	72.4±2 (12)	51.4±7.3 (8)	35.9±2.9 (8)	1.4
*lean lit-	40-49	114.1±2 (16)	270.8±46.2 (6)	172.3±19.0 (6)	1.5
termates	50-59	120.7±3 (12)	363.2±46.3 (6)	173.8±29.9 (6)	2.1
	70-79	102.6±3 (13)	476.3±40.0 (6)	153.3±12.1 (6)	3.1
NZO	27-33	54.2±2 (15)	52.8±7.5 (6)	34.7±6.8 (6)	1.5
	40-49	79.4±3 (10)	176.7±11.8 (9)	102.4±14.1 (8)	1.7
	60-69	99.0±3 (11)	382.0±52.0 (6)	193.2±11.8 (6)	1.4

mean ± SEM

The number of animals is indicated in parentheses

measured in fat pads of animals weighing 40-59 g and declines with increasing body weight. Consequently the ratio FFA/glycerol release increases in *obob* mice and remains nearly unchanged in NZO mice. In lean siblings and in NZO mice of the same weight the release of FFA and glycerol per fat cell is equal whereas the cell diameters are different. It is insignificantly greater than in adipose tissue of Swiss albino mice (Table 2).

Stimulated FFA and glycerol release in Swiss albino mice, lean littermates of obob mice and normal weight

NZO mice. In lean animals of the three strains studied (Fig. 3) the addition of lipolytic agents results in stimulation of FFA and glycerol release. The response to epinephrine and ACTH in lean littermates and NZO mice is slightly greater than in Swiss albino mice, whereas the stimulation by endogenous and exogenous cyclic AMP is equal in all strains.

FFA and glycerol release in obob mice of different weight and age. In *obob* mice lipolysis is markedly increased by the addition of lipolytic agents when compared to lean litters (Fig. 4). However, the stimulation of lipolysis is age-dependent. The greatest release of FFA is seen in mice weighing 40-49 or 70-79 g. Adipose tissue of *obob* mice weighing 50-59 g responds to lipolytic stimulation to a lesser extent. It may be noted that in this age group we found the largest fat cell diameters and divergent reactions to glucose load as shown before. Glycerol release is independent of age.

Stimulated FFA and glycerol release in NZO mice of different weight and age. Both basal and stimulated lipolysis of adipose tissue of NZO mice increases with increasing body weight (Fig. 5). The effect of DB 3,5-cyclic AMP, norepinephrine and ACTH is similar. However, theophylline exerts no significant lipolytic effect.

Discussion

It seems to be a trend of recent research in obesity to consider that even non-endocrine, so-called simple obesity is not a single entity but includes at least two

different types which can be characterized by differences in adipose tissue morphology. In man Preiss and coworkers [17] showed that in some patients the increment in adipose tissue mass is due to increasing cell size while in other forms of obesity there must be an additional increment in cell number. Salans and coworkers [20] and Jahnke and coworkers [10] came to the same conclusions in man and Hellman [5] proposed different types of similar characteristics in obese-hyperglycemic mice. As we have shown in NZO the size of the fat cell grows continuously and in proportion to

body weight. Since the volume of the cell is increased 17-fold, it can be calculated that in these animals the increase in body weight which is about 50 g, may be completely due to this process. The type of obesity in NZO mice may therefore be called hypertrophic obesity.

eous, the type of obesity in *obob* mice may therefore be called hyperplastic obesity.

It is of interest that these two types of obesity are associated with differences in adipose tissue metabolism. The hypertrophic form of obesity in NZO mice shows a nearly linear increment in spontaneous and stimulat-

Fig. 3. Effect of theophylline, 3,5 DB-cyclo AMP, norepinephrine and ACTH on release of free fatty acids (FFA) and glycerol (glyc.) by adipose tissue *in vitro*

I = Swiss albino mice (25–30 g)
 II = American obese-hyperglycemic mice (lean; 25–33 g)
 III = New Zealand obese mice (27–33 g)

Fig. 4. Effect of theophylline, 3,5 DB-cyclo AMP, norepinephrine and ACTH on release of free fatty acids (FFA) and glycerol (glyc.) by adipose tissue *in vitro* in American obese-hyperglycemic mice (*obob*)

By contrast in *obob* mice fat cell size and body weight are only correlated during the first months of age. When the maximal fat cell size is reached, the diameter of the fat cells in subcutaneous and perirenal adipose tissue remains constant (unpublished observation) in spite of an increase in weight and total body lipid content [2]. Since epididymal fat cells decrease not only in size but also in number [5, 6] this can only be understood if the number of cells in other locations increases. As the main deposition of fat is subcutan-

ed lipolysis with increasing fat cell volumes. It is noteworthy that the ratio FFA/glycerol release is not dependent on fat cell diameter and/or the nature of stimulation. It cannot be decided whether the sensitivity of lipolysis is a function of cell size, since other factors, due to changes of the hormonal and metabolic situation in the organism of the obese may also play a role in lipid mobilization. On the other hand, basal and stimulated lipolysis in *obob* mice does not exhibit such a correlation with either age or weight. Spontaneous

FFA release also increases but glycerol release remains unchanged with increasing weight. According to the current hypotheses decreased re-esterification may be

they were not observed when fat mobilization was related to nitrogen, due to the relatively higher nitrogen content of adipose tissue of *obob* mice [14, 16].

Fig. 5. Effect of theophylline, 3,5 DB-cyclo AMP, norepinephrine and ACTH on release of free fatty acids (FFA) and glycerol (glyc) by adipose tissue in vitro in New Zealand obese mice

Fig. 6. Epididymal fat cell size, spontaneous lipolysis and stimulated lipolysis with contribution to body weight in *obob* mice and NZO mice

postulated. However, it is also possible that an increased activity in glycerolkinase [23] may result in more pronounced intracellular re-utilization of glycerol. Similar results have been obtained when the basal FFA release was related to wet weight [25] but

In contrast to NZO mice stimulated lipolysis in *obob* mice is not correlated to cell size. The greatest lipolytic response is found in fat cells of submaximal diameter, whereas fat cells with maximal size exhibit the smallest response.

Since the changes in lipolytic response were observed after addition of norepinephrine or ACTH as well as theophylline or DB 3,5-cyclic AMP it may be concluded that the decreased lipolysis in animals weighing 50–59 g is due neither to a disturbed reaction of a first messenger with the reactor side [18] at the cell membrane nor to endogenous cyclic-AMP production but rather to a defect in the mechanism of activation of the inactive prolipase. In agreement with this, Lochaya and coworkers [15] observed a decreased lipase activity in adipose tissue in about 4 months old mice.

From the observation in *obob* mice it may thus be concluded, that factors other than cell size are responsible for the changing sensitivity to lipolytic agents. It seems noteworthy that the lipolytic response decreases simultaneously with fat cell proliferation. This occurs at the time when, for a short period, excessively high serum insulin levels can be observed. Although insulin resistance of glucose metabolism has been demonstrated in *obob* mice [21] this need not implicate a resistance of adipose tissue in lipolysis or possibly cell proliferation and it may be speculated that insulin is of importance to both phenomena.

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