Plasma Growth Hormone in the New Zealand Obese Mouse

R. G. LARKINS

University of Melbourne Department of Medicine, Royal Melbourne Hospital, Victoria, Australia

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Summary. Plasma growth hormone was measured by radioimmunoassay under basal conditions and after glucose administration in the New Zealand Obese (NZO) mouse and in a control strain. There was greater variability of plasma IRGH in the NZO mice than in the control strain under basal conditions, but there was no significant difference between the mean log plasma IRGH in the two strains. Moreover, in both strains rapid suppression of plasma IRGH occurred following glucose administration. It appears unlikely that pituitary hypersecretion of growth hormone accounts for the metabolic abnormalities observed in the NZO mouse.

L'hormone de croissance plasmatique chez la souris obèse de la Nouvelle-Zélande

Résumé. L'hormone de croissance immunoréactive (IRGH) plasmatique a été mesurée par une méthode radio-immunologique dans des conditions de base et après l'administration de glucose chez la souris obèse de la Nouvelle-Zélande (NZO) et aussi chez une souche de souris témoins. On a constaté plus de variabilité de IRGH plasmatique chez la souris NZO que chez la souche de souris témoins dans les conditions de base, mais il n'y avait pas de différence notable entre la moyenne logarithmique de IRGH plasmatique dans les deux souches de souris. D'ailleurs dans toutes les deux races il y avait une

suppression rapide de IRGH plasmatique après l'administration de glucose. Il paraît peu probable que l'hypersecretion pituitaire de l'hormone de croissance explique les anomalies métaboliques observées chez la souris NZO.

Das Plasma-Wachstumshormon bei der New Zealand Obese mouse

Zusammenfassung: Das Wachstumshormon im Plasma wurde unter Anwendung der Radioimmunomethode unter basalen Bedingungen und nach Zufuhr von Glucose bei der New Zealand obese mouse (NZO) und einer Kontrollzucht gemessen. Unter basalen Bedingungen bestand bei den NZO-Mäusen eine größere Variabilität des immunreaktiven Wachstumshormons im Plasma (IRGH) als bei der Kontrollzucht; es fand sich jedoch kein wesentlicher Unterschied des mittleren logarithmischen Plasma-IRGH bei den beiden Zuchten. Darüberhinaus erfolgte nach Zufuhr von Glucose bei beiden Zuchten eine schnelle Suppression des Plasma-IRGH. Es ist unwahrscheinlich, daß die pituitäre Hypersekretion des Wachstumshormons für die metabolischen Anomalien bei der NZO-Maus verantwortlich zu machen ist.

Key-words: NZO mouse, plasma growth hormone, suppression after glucose.

The New Zealand Obese (NZO) mouse is one of a number of strains of mice with genetically determined obesity, accompanied by carbohydrate intolerance, insulin resistance and hyperinsulinaemia. M. and F. Bielschowsky who were responsible for breeding this strain [1] suggested that the metabolic disorder might be due to excessive growth hormone activity [2]. More recently, Nakamura [13] and Herbai *et al.* [6, 7] have produced indirect evidence to support this hypothesis in the "KK" and "ob-ob" strains of obese hyperglycaemic mice respectively. So far, no direct measurements of plasma growth hormone have been reported in any of these mice. In this study, the plasma immunoreactive growth hormone (IRGH) was measured in the NZO mouse and in a control strain and the effect of administration of glucose on the plasma IRGH was determined.

Materials and Methods

NZO mice aged 7 to 42 weeks, and C₅₇B mice used as a control strain aged 7 to 36 weeks were obtained from the Walter and Eliza Hall Institute of Medical

Research, Melbourne. Mice from both strains were maintained under identical conditions with water and dog cubes, which contained fat 5%, protein 20%, carbohydrate 50% and added vitamins, available *ad libitum*.

Blood samples were obtained under light ether anaesthesia from the orbital sinus through heparinized 100 µl 'Microcap' pipettes. Between 100 and 400 µl of blood was obtained from each mouse and collected into iced tubes without further heparin. After centrifugation the plasma was stored at -15°C until assay. To allow for duplicate estimations and "blanks" for each sample, blood from littermates of the same sex was pooled for each growth hormone estimation.

The samples were taken after an overnight fast (6 p.m. to 10 a.m.), and after a 3 h fast (9 a.m. to 12 midday). Only adult mice (> 10 weeks) were used for these basal determinations. 18 groups of NZO mice (each group containing 2 to 4 littermates of the same sex), age range 14 to 36 weeks (mean 26 weeks) were studied after an overnight fast and 12 groups of C₅₇B mice age range 14 to 36 weeks (mean 27 weeks). The NZO and C₅₇B mice each contained equal proportions of males and females. After a 3 h fast, 35 groups

of NZO mice, age range 11 to 42 weeks (mean 24 weeks) and 17 groups of C₅₇B mice, age range 13 to 36 weeks (mean 24 weeks) were studied.

In other mice, the effect of the administration of glucose on plasma IRGH was determined. This test was performed on 7 different age groups of C₅₇B mice, age range 7 to 36 weeks (mean 20 weeks) and on 12 different age groups of NZO mice, age range 7 to 42 weeks (mean 27 weeks). Each group contained 2 to 4 littermates of the same sex. The test on each group was performed over 7 days, with two days between successive procedures. On day 1, blood was obtained after a 3 h fast. On day 3, after a 3 h fast, glucose 2 mg/g was injected intraperitoneally into each animal and blood obtained 5 min later. On days 5 and 7 a similar procedure was followed with blood being obtained at 20 and 40 min after the injection respectively. Thus on each day, the mice had only one anaesthetic and bleed. Control experiments were performed on 5 groups of NZO mice and 3 groups of C₅₇B mice using intraperitoneal administration of a similar volume of 0.9% saline, and otherwise identical conditions.

Blood sugar estimations were performed with a "Technicon Autoanalyser" using a ferricyanide method, adjusted to measure 25 μ l specimens of blood with an error of $\pm 10\%$ at 86 and 204 mg/100 ml.

Plasma IRGH was measured using a radioimmunoassay. Rat growth hormone (NIAMD — Rat GH-RP-1) was used as the standard. A more purified form of rat growth hormone (NIAMD-Rat GH-I-1) was labelled with ¹²⁵I in a method which was similar to that described by Hunter and Greenwood [8]. 10 μ g of rat growth hormone, 0.5 mCi of ¹²⁵I and 70.4 μ g of chloramine T were mixed in 0.3 M phosphate buffer, pH 7.6. 15 seconds later the iodination reaction was stopped by the addition of 240 μ g of sodium metabisulphite. A cellulose column was used for purification of the tracer, as this proved more satisfactory than Sephadex gel filtration. ¹²⁵I-labelled growth hormone was eluted with 20% acetone in 1% human serum albumin-veronal buffer, as described by Yalow and Berson [18]. Rat growth hormone antiserum obtained in monkeys was used (NIAMD-Anti-Rat GH serum 1) at 1 : 15000 concentration. All assays were performed in duplicate, using 50 μ l samples of mouse plasma. The standard curve was made up in phosphosaline-albumin buffer pH 7.6 with 50 μ l of human plasma added to each tube. The standard or samples and antiserum were incubated for 48 h at 4°C, then tracer was added and further incubation performed for 48 h. The final incubation volume was 500 μ l. Separation of free from bound tracer was performed using dextran-coated charcoal, as described by Jacobs [9], but modified so that 40 mg of charcoal was added to each tube. Correction for non-specific binding of tracer in the mouse plasma was made by including a "blank" without antibody for every plasma sample assayed.

The validity of the assay system was established by dilution and recovery experiments. Fig. 1 shows that

points obtained by diluting a pooled sample of mouse plasma with human plasma lie on the curve obtained using rat growth standard in human plasma in the range 5 to 50 ng/ml. In table 1, recoveries obtained

Fig. 1. Standard curve for rat growth hormone standard, showing that points obtained by diluting mouse plasma lie on this curve

● standard points
○ points obtained by diluting mouse plasma

Table 1. Values obtained for rat growth hormone standard added to a pooled sample of mouse plasma

Observed value ng/ml	Expected value ng/ml	Percentage recovery
89	112.5	79
59	62.5	94
41	37.5	109
26	28.5	91
17	22.5	76
15.5	17.5	89
12.5	14.5	85

after adding rat growth hormone standard to mouse plasma are shown. They ranged from 76% to 109% between 14.5 and 112.5 ng/ml. The intra-assay coefficient of variation was 10% at 12.5 ng/ml. The sensitivity of the assay routinely was 1 ng/ml or less. Non-specific binding in the system tended to be high, being 15 to 20% in human plasma, and 5 to 10% higher in mouse plasma.

The values quoted subsequently for plasma IRGH refer to the weight of rat growth hormone standard preparation equivalent immunologically to the mouse growth hormone.

Results

1. Plasma IRGH in the NZO mice and in the control strain. For the age range studied, age did not affect plasma IRGH, as shown in Fig. 2. Mice of different ages were therefore considered together.

The plasma IRGH values obtained after an overnight fast are shown in Fig. 3 and those obtained after a three-hour fast are shown in Fig. 4. The variance was

Fig. 2. Plasma IRGH after a three-hour fast plotted against age. Each point represents the plasma IRGH in a pooled sample from 2–4 littermates of the same sex

For NZO mice $r = -0.28$, $p > 0.05$. For C₅₇B mice $r = -0.27$, $p > 0.05$. A similar lack of association between plasma IRGH and age was found using values obtained after an overnight fast

significantly greater in the NZO mice under both conditions ($F = 15$, $p < 0.01$, and $F = 3.8$, $p < 0.01$ for the overnight fast and three-hour fast respectively, where F is the variance ratio). It can be seen that there were some conspicuously high values obtained for a few of the NZO mice, but that the majority had similar plasma IRGH levels to those in the C₅₇B mice. For statistical comparison, logarithmic conversion was used to standardize the variances and to approximate a normal distribution. The t test for unpaired data was then applied, and there was no statistically significant difference between the mean log values of plasma IRGH for the NZO and C₅₇B mice, both after an over-

Fig. 3. Plasma IRGH after an overnight fast. Each point represents the plasma IRGH in a pooled sample from 2–4 littermates of the same sex

Fig. 4. Plasma IRGH after a three-hour fast. Each point represents the plasma IRGH in a pooled sample from 2–4 littermates of the same sex

night fast and after a three-hour fast ($t = 0.69$ and $t = 1.84$ respectively).

The mean blood sugar concentration ($\pm$ SEM) for

NZO mice after an overnight fast was 105.4 ± 3.6 mg/100 ml and this value was slightly higher than the mean concentration of 91.8 ± 3.3 mg per 100 ml for the C₅₇B mice ($t = 2.73$, $p < 0.01$). After a 3 h fast, the mean concentration was 152.9 ± 3.8 mg/100 ml compared with 140.9 ± 2.9 mg/100 ml for the C₅₇B mice ($t = 3.55$, $p < 0.02$).

2. Effect of administered glucose on plasma IRGH.

The mean plasma IRGH for the 7 groups of C₅₇B mice and 12 groups of NZO mice following glucose administration, is shown in figure 5, and the mean blood sugar levels at the corresponding times are shown in Fig. 6. It is apparent that in both strains there is a rapid suppression of plasma IRGH apparent 5 min after

Fig. 5. Plasma IRGH following intraperitoneal glucose (2 mg/g). The mean values and SEM are shown

○ — ○ NZO
○ - - - ○ C₅₇B

glucose administration. The suppression was similar in both strains though it tended to be more complete and prolonged in the NZO mice. A fall in plasma IRGH of at least 33%, and in most cases greater than 50%, was observed without exception in every age group tested at 5 or 20 min after the glucose injection. The plasma IRGH in the NZO mice at 5 min, 20 min and 40 min after the intraperitoneal glucose load was significantly lower than prior to the glucose ($p < 0.02$, < 0.01 , < 0.01 respectively — paired t test). The rise in blood sugar was significantly greater and more

Fig. 6. Blood sugar following intraperitoneal glucose (2 mg/g). The mean values and SEM are shown

○ — ○ NZO
○ - - - ○ C₅₇B

Fig. 7. Plasma IRGH for individual groups of mice following the injection of 0.9% saline

● — ● NZO
● - - - ● C₅₇B

sustained in the NZO mice than in the C₅₇B mice with this glucose load (Fig. 6).

In the control experiments in which 0.9% saline was injected instead of glucose, wide fluctuations were seen in the individual plasma IRGH levels (Fig. 7) despite relatively stable blood sugar levels (Fig. 8).

However, there was no tendency towards suppression of plasma growth hormone at 5 and 20 min following the saline injection in either strain. In fact, in the $C_{57}B$ mice there was a rise in plasma IRGH at 20 min, followed by a fall at 40 min. In both strains, the

Fig. 8. Blood sugar for individual groups of mice following the injection of 0.9% saline

● — ● NZO
● - - - ● $C_{57}B$

plasma IRGH 20 min after the glucose was significantly lower than after the saline injection ($p < 0.001$ for NZO mice, $p < 0.01$ for $C_{57}B$ mice).

Discussion

Obesity [1, 4], carbohydrate intolerance [4, 10], hyperinsulinaemia [10, 15] and insulin resistance [2, 4] have been well documented in the NZO mouse. However the cause and mechanism of these abnormalities remain uncertain. The metabolic disorder appears similar to that in the ob-ob strain [16] and in the KK strain [13]. Herbai *et al.* have recently reported [7] that there is an increased rate of incorporation of labelled sulphate into the costal cartilage of obese-hyperglycaemic mice of the ob-ob strain. They have suggested that this may be associated with increased growth hormone activity, but could not exclude raised plasma insulin as a possible cause. Herbai [6] has also shown that hypophysectomy reversed the weight gain of these animals, but whether this was an effect on growth hormone production or on hypothalamic appetite regulatory centres is unknown. Nakamura *et al.* [13] reported increased pituitary growth hormone content using gel electrophoresis in the KK strain, but no extrapolation can be drawn to the plasma level of growth hormone.

The validity of the radioimmunoassay technique described for mouse growth hormone using antisera to rat growth hormone and rat growth hormone tracer has been established. Non-specific binding in the system was quite high, but the problem of large and variable degrees of non-specific binding in different plasmas, recently described for rat serum using the dextran-charcoal method [12] was not encountered. The specificity of an assay system using rat growth hormone tracer and antiserum has been assessed by other workers [3] and no significant binding was demonstrated with other rat pituitary hormones. There was no significant sex or age difference in plasma IRGH within the strains under the conditions studied, so males and females of different ages were considered together. A possible effect of repeated trauma or anaesthesia [14, 17] was avoided by performing bleeds at different periods after glucose on separate days with the sample taken within 45 sec of the commencement of the ether anaesthetic. The suppression in plasma growth hormone was shown to be due to the hyperglycaemia and not to the experimental procedure, by control experiments using intraperitoneal saline injections, where no similar early suppression of plasma IRGH was observed. The wide variation in plasma IRGH following saline reflects the lability of growth hormone secretion rate, and could not be accounted for by changes in blood sugar levels.

Apart from significantly greater variability in the NZO mice and some occasionally very high values, there was little difference in the levels of plasma IRGH in the two strains of mice. Blood sugar levels under basal conditions were slightly higher in the NZO mice than in the $C_{57}B$ mice, but the levels were comparable and the slight difference would not be expected to influence the plasma IRGH. The reason for the greater variability in the plasma IRGH in the NZO mice is not clear, although a large degree of variability is also noted in body weight and glucose tolerance in different animals of this strain [4]. A wide range of fluctuations in plasma IRGH has been observed in mice by other workers [11], and probably represents spontaneous bursts of growth hormone secretion. More significantly, plasma IRGH was suppressed dramatically following glucose administration in both strains of mice. This phenomenon has recently also been described in rats [17], and contrasts with the reported lack of rise of plasma IRGH in response to hypoglycaemia in rats [5] and mice [11]. Thus plasma IRGH responds in a qualitatively identical manner to administration of glucose and induced hyperglycaemia in both strains. Although this stimulus is an artificial one, it tests the competence of one homeostatic mechanism and reveals it to be functioning normally. This, together with the comparable basal levels, makes it most unlikely that pathological secretion of growth hormone from the pituitary is responsible for the metabolic abnormalities in the NZO mouse.

However, immunological activity does not neces-

sarily parallel biological activity [5], and the possibility that some biologically active but immunologically inactive fragment of growth hormone is being produced in excess cannot be completely excluded. Moreover, results in the NZO strain of obese-hyperglycaemic mice cannot necessarily be extended to other strains, although the metabolic abnormalities appear similar.

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Dr. R.G. Larkins
University of Melbourne
Dept. of Medicine
Royal Melbourne Hospital
Victoria, Australia