Congenitally Impaired Hormone Sensitivity of the Adipose Tissue of Spontaneously Diabetic Mice, KK

Validity of Thrifty Genotype in KK Mice

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Summary. Adipose tissue of KK mice was less sensitive to insulin in its stimulatory action on glucose oxidation or its inhibitory action on lipolysis, and to epinephrine in its stimulatory action on lipolysis as compared to that of C57BL mice. A lack of appreciable increase in plasma NEFA in response to fasting of the KK mice might be caused by the impaired response of lipolysis to the hormones. — Adipose tissue of KK mice was also less sensitive to insulin-like action of concanavalin A, ouabaine or omission of K^+ in the medium on glucose oxidation or lipolysis. Lipolysis in response to theophylline and/or DcAMP was less marked in the tissue of KK mice. The mean diameter of the adipocytes was larger in KK than in C57BL mice. In the experiments using adipocytes with the same diameter, however, the cells of KK mice were less sensitive to the hormones than those of C57BL. — With respect to tissue sensitivity to the hormones, the mean diameter of adipocytes and the response of plasma NEFA to fasting, the F_1 -hybrid mice of KK and C57BL showed values between those of the parental strains.

Yellow hybrid (genetically obese F_1 -hybrid) mice showed higher sensitivity to insulin in the adipose tissue, more remarkable response of plasma NEFA to fasting as compared with KK mice, although hypertrophy of adipocytes was more pronounced in the yellow hybrid mice. — These findings suggest that insulin insensitivity is associated with epinephrine insensitivity in adipocytes of KK mice; both appear to be subjected to genetic factor(s) *per se* rather than hypertrophy of the adipocytes. Furthermore, insensitivity to both hormones in the KK mice appears to be caused by a common defect in cellular process other than the hormone receptor systems. These genetically determined abnormalities of adipocytes may result in fat-storage metabolism through hyperinsulinemia; this is very similar to the metabolic profile due to thrifty genotype in human diabetes as proposed by Neel.

Key words: Insulin, epinephrine, lipolysis, glucose metabolism, fat cell, adipose tissue, plasma NEFA, KK, yellow KK, C57BL, diabetes, genetics.

We have previously demonstrated that the KK mice were more susceptible to diabetogenic action by obesity, induced by goldthioglucose, high calorie diets or the yellow obese gene (A^y), than ICR, C57BL or hybrid mice of KK and C57BL [1, 2, 3]. Thus, KK strain appeared to carry a genetically determined predisposition to diabetes.

Before the onset of diabetes and/or obesity, glucose intolerance and insulin insensitivity were observed in the KK mice [4], and these characteristics were under a common genetic control, presumably polygenic [1, 5]. Furthermore, ICR mice showed higher susceptibility to diabetogenic action by goldthioglucose-induced obesity than C57BL mice [3], and ICR mice, prior to the onset of obesity, were less tolerant to glucose and less sensitive to insulin than C57BL mice. Therefore, glucose intolerance and insulin resistance appeared to be markers indicating predisposition to diabetes.

In the present studies, adipose tissue metabolism was examined with varying genetic or biochemical backgrounds in order to relate its involvement in the pathophysiology of diabetes in the KK mice.

Materials and Methods

Animals

Mice were bred in our laboratories as described previously [1]. KK and yellow KK mice were offspring of female KK and male yellow KK mice. F_1 -hybrid mice and yellow hybrid mice were offspring of female C57BL/6J and male yellow KK mice. Female yellow KK mice were not used as breeders because of high incidence of infertility. Male mice of each group were used for experiments at 8 to 10 weeks of age. They were fed a lab chow (CE2, Clea Japan, Tokyo) and water *ad libitum*.

Response of Plasma NEFA to Fasting

The mice were fasted for 20 h (5:00 p.m. to 8:00 a.m.), an equal number were fed *ad lib* and served as controls. Blood was taken by heart puncture from mice anesthetized by ether at 8 to 9 a.m. Plasma glucose and NEFA were determined by glucose oxidase method [6] and Novak's method [7] respectively.

Experiments with Adipose Tissue or Adipocytes

For assay of lipolysis in epididymal adipose tissue, 80 mg fat pad was incubated in 1 ml of Krebs-Ringer

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bicarbonate buffer containing 20 mg of bovine albumin (Fraction V). Flasks were gassed with 95% O₂:5% CO₂ mixture and shaken at 80 strokes/min at 37° C for 120 or 180 min. NEFA released into the medium was extracted and measured [8, 6].

For assay of glucose oxidation, 80 mg of fat pad was incubated in 1 ml of the same buffer containing 20 μ moles of glucose (glucose-1-¹⁴C, 0.1 μ Ci). The experimental procedures were described in our previous paper [3].

Adipocytes were obtained from epididymal fat pads by digestion with collagenase [9]. Fractionation of adipocytes according to the cell size was performed according to Björntorp's method [10]. The cells were fixed in trichloroacetic acid-glutaraldehyde [11] and the cell diameter was measured by micrometer [12].

The cells were suspended in 1 ml of Krebs-Ringer bicarbonate buffer containing 40 mg albumin in a plastic vial. The procedures for assay of lipolysis were the same as described above. For assay of glucose oxidation, the above medium contained 2.8 μ moles glucose (glucose-1-¹⁴C, 0.5 μ Ci). The incubation procedures were the same as described previously [3].

Results

Impaired Responses of Adipose Tissue to Hormones

As previously described, glucose oxidation and lipolysis were less sensitive to insulin and epinephrine, respectively, in adipose tissue of KK mice than in that of C57BL mice (Fig. 1). This figure indicates a positive correlation between sensitivity to both hormones in mouse adipose tissue from KK and C57BL mice.

Responses of adipose tissue metabolism to various stimuli are summarized in Tables 1, 2 and 3. Concanavalin A or omission of K⁺ in the medium stimulated glucose oxidation. These insulin-like actions were less pronounced in the tissues of KK mice (Table 1). The adipose tissue of KK mice was less sensitive to the insulin-like actions of ouabain and K⁺-omission (Table 2). An inhibitory action on epinephrine-stimulated lipolysis by Con A or insulin was also less remarkable in adipose tissue or adipocytes of KK mice (Tables 2 and 3).

A stimulatory action by DcAMP or theophylline was less pronounced in the tissue and cells of KK mice (Tables 2 and 3). Combination of theophylline with epinephrine or DcAMP stimulated lipolysis; the cells of KK mice responded to these stimuli to less extent as compared with those of C57BL mice (Table 3).

Genetic Control of Adipose Tissue Metabolism

In our previous work [1], it was confirmed that KK mice were less tolerant to glucose and less sensitive to insulin than C57BL mice. Furthermore, F₁-hybrid mice of KK and C57BL mice showed intermediate response regarding these clinical parameters.

Plasma NEFA levels after overnight fasting were measured in mice with different genotypes (Fig. 2). In

KK mice, the NEFA level was higher in the fed state but lower in the fasted state as compared with C57BL mice at similar dietary states. No appreciable increase of the NEFA level due to fasting was observed in KK mice. F₁-hybrid mice showed intermediate levels of plasma NEFA in the fed and fasted states, and the change of NEFA levels in response to fasting also fell between those of the parental strains.

Fig. 1. Relationship between insulin and epinephrine sensitivity in mouse adipose tissue. The hormone sensitivity is expressed as percent increase of the basal activity of glucose-1-¹⁴C or NEFA release due to insulin (1 mU/ml) or epinephrine (0.1 μ g/ml = 3×10^{-7} M). Experimental conditions as described in the text

Yellow (obese) hybrid mice showed a significantly lower level of NEFA in the fasted state, but not in the fed state; consequently, there was a lower response to fasting in the yellow hybrids than those in the lean controls, F₁-hybrid. Although yellow (obese) KK (KK-A^y) mice showed hyperglycemia (higher than 300 mg%), their plasma NEFA level and its change in response to fasting was not different from that of their lean controls, the KK mice.

Response of lipolysis to epinephrine was examined in the adipose tissue from KK, C57BL and F₁-hybrid (Fig. 3); the values, expressed as per cent of the basal activity, were 195% for C57BL, 71% for F₁-hybrid and 59% for KK mice, respectively.

Responses of glucose oxidation to insulin, together with the size of the adipocytes were examined (Fig. 4). At 100 μ U/ml insulin, the response of adipose tissue of

F₁-hybrid mice was intermediate of those of the parental strains. The tissues from yellow obese mice, such as yellow hybrid and yellow KK, were less sensitive to insulin than those from their respective lean controls. The sensitivity decreased in the following order: C57BL, F₁-hybrid, yellow obese hybrid, KK and

(Fig. 5). A negative correlation between the cell size and sensitivity to insulin or epinephrine was evident in the cells of C57BL mice, but similar relationship was not present in those of KK mice. In addition, KK adipocytes were less sensitive to the hormones than the C57BL adipocytes of similar sizes, i.e. 50–70 μ .

Table 1. *Glucose-1-¹⁴C oxidation by adipose tissue or adipocytes of C57BL and KK mice*

Experiment	Addition	N	Relative activity	
			C57BL	KK
1 adipose tissue	None	5	100 (0.150 $\pm$ 0.017) ^a	100 (0.0899 $\pm$ 0.0200) ^a
	None (K ⁺ -free)	5	150 $\pm$ 7	119 $\pm$ 5 ^b
2 adipocytes	None	6	100 (0.214 $\pm$ 0.014) ^c	100 (0.143 $\pm$ 0.010) ^c
	con A ^e (5 $\times$ 10 ⁻⁸ M)	6	156 $\pm$ 20	120 $\pm$ 2
	con A (4 $\times$ 10 ⁻⁷ M)	6	207 $\pm$ 9	149 $\pm$ 3 ^d
	con A (1 $\times$ 10 ⁻⁶ M)	6	196 $\pm$ 1	144 $\pm$ 6 ^d

^a μ moles/100 mg tissue/90 min (Mean $\pm$ S.E.M.)

^b $P < 0.01$ (KK vs. C57BL)

^c nmoles/mg triglyceride/90 min

^d $P < 0.005$ (KK vs. C57BL)

^e concanavalin A

Table 2. *NEFA-release by adipose tissue of C57BL and KK mice*

Experiment	Addition	M	N	Relative activity	
				C57BL	KK
1	None	—	5	100 (1.01 $\pm$ 0.13) ^a	100 (0.570 $\pm$ 0.165) ^a
	None (K ⁺ -free)	—	5	50 $\pm$ 0.6	76 $\pm$ 9 ^b
2	None	—	4	100 (0.775 $\pm$ 0.045) ^a	100 (0.572 $\pm$ 0.111) ^a
	Ouabain	1 $\times$ 10 ⁻⁴	4	55 $\pm$ 4	76 $\pm$ 5 ^b
3	None	—	5	— (0.735 $\pm$ 0.059) ^a	— (0.679 $\pm$ 0.095) ^a
	Epi	1 $\times$ 10 ⁻⁶	5	100 (1.94 $\pm$ 0.28) ^a	100 (1.18 $\pm$ 0.13) ^a
	Epi	1 $\times$ 10 ⁻⁶	5	63 $\pm$ 7	92 $\pm$ 5 ^c
	+Ins (10 mU/ml)	—	—	—	—
4	None	—	5	100 (0.797 $\pm$ 0.062) ^a	100 (0.556 $\pm$ 0.042) ^a
	DcAMP	1 $\times$ 10 ⁻⁴	5	130 $\pm$ 15	116 $\pm$ 14
	DcAMP	1 $\times$ 10 ⁻³	5	151 $\pm$ 12	117 $\pm$ 9 ^d
	DcAMP	1 $\times$ 10 ⁻²	5	204 $\pm$ 24	123 $\pm$ 14 ^b
5	None	—	5	100 (0.657 $\pm$ 0.036) ^a	100 (0.599 $\pm$ 0.050) ^a
	Theo	1 $\times$ 10 ⁻⁵	5	147 $\pm$ 12	103 $\pm$ 2 ^c
	Theo	1 $\times$ 10 ⁻⁴	5	180 $\pm$ 21	131 $\pm$ 10
	Theo	1 $\times$ 10 ⁻³	5	314 $\pm$ 17	174 $\pm$ 11 ^e
6	None	—	5	100 (0.672 $\pm$ 0.064) ^a	100 (0.542 $\pm$ 0.067) ^a
	DcAMP	1 $\times$ 10 ⁻³	5	213 $\pm$ 32	117 $\pm$ 9 ^b
	Theo	1 $\times$ 10 ⁻³	5	298 $\pm$ 17	181 $\pm$ 10 ^e
	DcAMP + Theo	1 $\times$ 10 ⁻³	5	358 $\pm$ 27	287 $\pm$ 25

^a μ eq/100 mg tissue/120 min (Mean $\pm$ S.E.M.)

^b $p < 0.025$ (KK vs. C57BL)

^c $p < 0.01$ (KK vs. C57BL)

^d $p < 0.05$ (KK vs. C57BL)

^e $p < 0.005$ (KK vs. C57BL)

Abbreviations: Ins (insulin), Epi (epinephrine), DcAMP (dibutyryl cyclic AMP), Theo (theophylline), con A (concanavalin A), NEFA (non-esterified fatty acid)

yellow KK mice. On the other hand, the mean cell size of the tissue increased in the following order: C57BL, F₁-hybrid, KK, yellow KK and yellow obese hybrid. Therefore, the lowered sensitivity of KK mice could not be accounted for by hypertrophy of the adipocytes.

The sensitivity to epinephrine or insulin was examined with isolated adipocytes of varying cell size

Discussion

In a series of experiments including the present studies [1, 4], we demonstrated that KK mice without hyperglycemia carry three clinical abnormalities: (i) glucose intolerance, (ii) insulin resistance and (iii) an impaired response of plasma NEFA to fasting. These

syndromes appear to be governed by a common inheritance mode.

The impaired sensitivity to insulin or epinephrine may be responsible for the metabolic abnormalities observed *in vivo*. For example, the reduction of insulin action on adipose tissue, as observed in the studies of glucose oxidation *in vitro*, may be a causal factor for either glucose intolerance or insulin resistance in the KK mice. The lowered response of lipolysis to insulin and epinephrine may result in the impaired response of plasma NEFA to fasting.

sensitivity in the adipose tissue and a more remarkable response to fasting in plasma NEFA levels than KK mice, although the mean cell size of the adipose tissue was significantly larger in the yellow (obese) hybrid mice. Furthermore, the adipocytes from KK mice were less sensitive to either insulin or epinephrine than C57BL adipocytes of similar sizes. The impaired sensitivity to the hormones, is therefore, more likely determined by genetic factor(s) carried in the KK strain than simply by the change in cellularity.

Impaired response of lipolysis to adipokinetic

Table 3. NEFA-release by adipocytes of C57BL and KK mice

Experiment	Addition	M	N	Relative activity	
				C57BL	KK
1	None	—	5	— (7.53 ± 0.78) ^a	— (3.70 ± 0.34) ^a
	Epi	1 × 10 ⁻⁶	5	100 (51.7 ± 5.3) ^a	100 (9.46 ± 1.47) ^a
	Epi +	1 × 10 ⁻⁶	5	75 ± 5	95 ± 2 ^b
	Ins (10 mU/ml)	—			
2	None	—	6	— (9.92 ± 0.38) ^a	— (7.18 ± 0.67) ^a
	Epi	1 × 10 ⁻⁶	6	100 (60.0 ± 2.6) ^a	100 (22.4 ± 3.14) ^a
	Epi	1 × 10 ⁻⁶	6	67 ± 4	92 ± 2 ^c
	+ Con A	5 × 10 ⁻⁸			
	Epi	1 × 10 ⁻⁶	6	67 ± 1	88 ± 1 ^c
	+ Con A	4 × 10 ⁻⁷			
	Epi	1 × 10 ⁻⁶	6	68 ± 1	88 ± 1 ^c
3	+ Con A	1 × 10 ⁻⁶			
	None	—	6	100 (7.16 ± 0.57) ^a	100 (3.51 ± 0.87) ^a
	DcAMP	1 × 10 ⁻⁴	6	186 ± 18	108 ± 5 ^c
	DcAMP	1 × 10 ⁻³	6	213 ± 43	111 ± 11 ^d
4	DcAMP	1 × 10 ⁻²	6	328 ± 19	119 ± 10 ^c
	None	—	6	100 (7.04 ± 0.67) ^a	100 (3.29 ± 0.25) ^a
	Theo	1 × 10 ⁻⁵	6	214 ± 15	113 ± 5 ^c
	Theo	1 × 10 ⁻⁴	6	453 ± 25	223 ± 26 ^c
5	Theo	1 × 10 ⁻³	6	810 ± 40	475 ± 41 ^c
	None	—	6	100 (8.51 ± 0.94) ^a	100 (3.26 ± 0.23) ^a
	Epi	3 × 10 ⁻⁷	6	360 ± 44	186 ± 29 ^b
	DcAMP	1 × 10 ⁻³	6	234 ± 38	151 ± 3 ^d
	Theo	1 × 10 ⁻⁵	6	190 ± 21	113 ± 5 ^c
	Epi	3 × 10 ⁻⁷	6	546 ± 77	338 ± 38 ^d
	+ Theo	1 × 10 ⁻⁵			
	DcAMP	1 × 10 ⁻³	6	369 ± 52	218 ± 15 ^e
	+ Theo	1 × 10 ⁻⁵			

^a neq/mg triglyceride/120 min

^b $p < 0.01$ (KK vs. C57BL)

^c $p < 0.005$ (KK vs. C57BL)

^d $p < 0.05$ (KK vs. C57BL)

^e $p < 0.025$ (KK vs. C57BL)

Abbreviations — See Table 2

The present studies indicated a negative correlation between the cell size and insulin or epinephrine sensitivity in adipocytes from C57BL mice. The mean cell size of adipose tissue increased in the following order: C57BL, F₁-hybrid and KK mice; whereas insulin sensitivity of adipose tissue decreased in the same order. It appears, therefore, that the change in cellularity may be the cause of hormone insensitivity in the KK mice.

However, this postulation was not valid in the KK mice due to the following two observations: Firstly, the yellow (obese) hybrid mice showed higher insulin

hormones and insulin insensitivity have also been observed in other genetically diabetic and/or obese mice [13–18]. It has been suggested that an impaired system of cAMP formation is responsible for epinephrine insensitivity in the NZO mice [19] and reduced number of receptor site leads to insulin insensitivity in the *ob/ob* mice [24]. However, it is not likely that impaired hormone sensitivity in the KK mice arises from similar mechanisms. Lipolysis by adipose tissue from KK mice was less sensitive to DcAMP, theophylline or combination of theophylline with epinephrine or DcAMP. Furthermore, glucose oxidation and

lipolysis by adipose tissue or adipocytes from KK mice were less sensitive to insulin-like actions of Con A, ouabain or omission of K^+ in the medium. The positive correlation between insulin and epinephrine sensitivity of mouse adipose tissue suggests the participation of

Fig. 2. Plasma NEFA level in fasted or fed state. Open bar: fed state. Shaded bar: fasted state. Number in parenthesis indicates number of mice. Mean $\pm$ S.E.M.

Fig. 3. NEFA release by adipose tissue in the presence or absence of epinephrine ($0.1 \mu\text{g/ml} = 3 \times 10^{-7}\text{M}$). Experimental conditions as described in the text. Open bar: absence of epinephrine. Shaded bar: presence of epinephrine. Number of parenthesis indicates number of experiments. Mean $\pm$ S.E.M.

common processes. It is therefore, postulated that impaired sensitivity of KK mice to both hormones are caused by common defects in the adipocytes, and not by abnormalities in the respective receptor sites for the hormones.

Previously we reported a positive correlation between hepatic lipogenic activity and the plasma insulin level in KK and obese KK mice, indicating a normal response of hepatic lipogenesis to insulin [20, 21]. Predisposition to diabetes in the KK mice may arise from a normal response to insulin in the liver concomitant with insulin insensitivity in the adipose tissue. Insulin insensitivity in the adipose tissue is likely to induce hyperinsulinemia due to a compen-

	N	Basal Glucose Oxidation (μ moles/g tissue/90 min.)	Size of adipocyte (μ)
C57BL	10	2.05 ± 0.14	47.0 ± 1.6
Hybrid	5	1.32 ± 0.10	56.6 ± 0.7
KK	12	1.08 ± 0.90	60.4 ± 0.8
Yellow Hybrid	7	1.21 ± 0.06	81.1 ± 1.0
Yellow KK	10	1.15 ± 0.04	75.2 ± 1.4

Fig. 4. Insulin sensitivity of adipose tissue from mice with different genotypes. Experimental procedures as described in the text. Mean $\pm$ S.E.M.

satory mechanism of the islets; hyperinsulinemia, in turn, may lead to obesity through activation of hepatic lipogenesis. Obesity may further reduce the genetically controlled hyposensitivity of the adipose tissue to the extent that hyperglycemia and severe hyperinsulinemia ensue. Thus, a vicious cycle is established between pancreatic islets and peripheral tissues with a mediating action by liver. This postulated sequence of metabolic events is very similar to the metabolic profile

caused by thrifty genotype in human diabetes proposed by Neel [22].

It has been demonstrated that the development of diabetes and/or obesity in KK mice requires other factors than the diabetic gene(s) [1, 4]. Either genetically or experimentally induced obesity was able to

Fig. 5. Relationship between adipocyte diameter and insulin or epinephrine sensitivity. Adipocytes were fractionated according to the cell diameter. Experimental conditions as described in the text. Results are expressed as percent increase of the basal activity of glucose-1-¹⁴C oxidation or NEFA-release due to insulin (1 mU/ml) or epinephrine (0.1 μg/ml = 3×10^{-7} M). Open circle: C57BL, Closed circle: KK

cause hyperglycemia in the KK mice, accompanied by severe hyperinsulinemia, further lowering insulin sensitivity in the adipose tissue and activating lipogenesis in the liver [20, 23]. This sequence of events again supports the postulation that the above mentioned vicious cycle is involved in the pathogenesis in the KK mice.

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