FULL PAPER *Internal Medicine*

Insulin Responses to Administrations of Amino Acids and Fatty Acids in Healthy Cats

Kyoji YASUDA[1)], Satoshi TAKASHIMA[1)], Mitsuru TAKAGI[1)], Naohito NISHII[2)], Yasunori OHBA[1,3)] and Hitoshi KITAGAWA[1,3)]*

[1)]*Department of Veterinary Clinical Veterinary Medicine, United Graduate School of Veterinary Science, Gifu University, 1–1 Yanagido, Gifu 501–1193,* [2)]*Laboratory of Veterinary Pharmacotherapeutics, Faculty of Agriculture, Tottori University, Tottori 680–8553 and* [3)]*Department of Veterinary Medicine, Faculty of Applied Biological Sciences, Gifu University, 1–1 Yanagido, Gifu 501–1193, Japan*

(Received 24 September 2010/Accepted 20 May 2011/Published online in J-STAGE 3 June 2011)

ABSTRACT. In order to compare the stimulation ability of insulin secretion, we determined changes in plasma glucose and insulin concentrations after intravenous administration of various amino acids and essential fatty acids in clinically healthy adult cats. Plasma glucose concentrations were within the normal ranges after injection of amino acids and fatty acids. Plasma insulin concentrations increased rapidly 2 to 4 min after injection of arginine, then decreased to the basal levels at 20 min in all five cats. Insulin peak responses were significantly greater in arginine injections than in normal saline ($P<0.01$). Areas under the curve (AUC) of plasma insulin concentrations from 0 to 10 min after injection of arginine were significantly larger than after injection of normal saline ($P<0.01$) and glucose ($P<0.05$). Increases in AUC of plasma insulin concentration from 0 to 60 min were observed after injection of arginine, leucine, alanine, and fat emulsion. Arginine had a strong insulinotropic effect, and leucine, alanine, and fatty acids had weak ones. Besides, valine, methionine, taurine and glutamine had no stimulant activity of insulin. Given the risk of glucose toxication and required time for testing, the intravenous arginine tolerance test may be useful for estimation of insulin responses in cats.

KEY WORDS: amino acid, arginine, fatty acid, feline, insulin secretion.

J. Vet. Med. Sci. 73(10): 1281–1286, 2011

Insulin response in cats has been estimated by intravenous glucose tolerance test [1, 3, 16, 17]. High-dose infusion of glucose, however, poses a great risk of developing glucose toxication in cats with obesity or diabetes mellitus. Because of the deficit of glucokinase, the glucose clearance time is longer in cats than in humans [13, 21] and dogs [11, 22]. Therefore, the intravenous glucose burden is hard to use to estimate insulin secretory property in cats with obesity or diabetes mellitus.

Arginine is also known as one of the nutrients which stimulates insulin secretion in humans [7, 9, 18], mouse [8, 10] and cattle [24]. Also in cats, arginine induced a secretory response of insulin in perfused pancreas cells [5]. In cats, insulin and glucagon responses were induced by high-dose intravenous injection of arginine [12]. In other amino acids, alanine and leucine reportedly induced insulin secretion in mouse pancreatic β-cells [8, 10]. Other than amino acid, fatty acid induced a secretory response of insulin in humans [9]. However, insulin secretory stimulation by amino acids other than arginine and fatty acids has not been examined in cats. In consideration of previous studies [2, 9], the glucogenic amino acids (valine, methionine), the essential amino acid only in cats (taurine) and a non-essential amino acid playing an important role in the energy metabolism (glutamine) may have the possibility of stimulating insulin secretion in cats.

To compare the insulin stimulation ability of these amino acids and fatty acids in healthy cats, we determined changes in plasma glucose and insulin concentrations after intravenous administrations of various essential and non-essential amino acids and essential fatty acids.

* CORRESPONDENCE TO: KITAGAWA, H., Department of Veterinary Medicine, Faculty of Applied Biological Sciences, Gifu University, 1–1 Yanagido, Gifu 591–1193, Japan.
e-mail: hkitagaw@gifu-u.ac.jp

MATERIALS AND METHODS

Animals: Nine clinically healthy cats (five males and four females) were used. They were all mixed-breed, 3 to 7 years old in age, 2.5 to 5.4 kg in body weight, and 5 to 6 in 9-point scale body condition score (BCS) [6]. Animals had been acclimated to the laboratory before the study, and handled gently to eliminate any discomfort or stress. The study was conducted in a manner consistent with the Gifu University Guidelines for Animal Experimentation (Approved No. 08073).

Substances examined: Arginine, alanine, leucine, valine, methionine, taurine and glutamine were used as amino acids. A fat emulsion formulation for intravenous injection (20% Intralipos, Otsuka Pharmaceutical Factory Inc., Tokushima, Japan) was used as a fatty acid mixture. The formulation contains linoleic acid (53%), olenic acid (24%), palmitin acid (12%), linolenic acid (7%) and stearin acid (4%). Amino acids were dissolved with distilled water, and filtrated by a 0.20 μm cellulose acetate filter sterilization system (DISMIC-13cp, Advantec, Tokyo, Japan). Solutions prepared were administered intravenously to cats for 30 sec. Doses of amino acids were designed to load up to 0.1 g arginine/kg of body weight on a molar basis according to the previous study [12]. As the negative and positive controls, a normal saline (1 m*l*/kg of body weight) and 50% glucose solution (1 m*l*/kg of body weight) were used, respectively.

Blood sampling: Blood samples after injection of normal

saline and glucose solution were collected in three cats, and other samples were collected in five cats. After withdrawal of food for 12 hr, fasting blood samples were collected from the jugular or femoral veins with a needle syringe in friendly cats. In volatile cats, blood samples were collected via an intravenous catheter placed more than 12 hr before the tolerance test. The catheter was inserted into the jugular vain under anesthesia by diazepam (Horizon, Astellas Pharmaceutical Inc., Tokyo, Japan) and ketamine hydrochloride (Ketamine Injection, Fujita Pharmaceutical Inc., Tokyo, Japan). Blood collections were carried out immediately before and 2, 4, 6, 8, 10, 20, 30, 45 and 60 min after injection of the substance. Plasmas separated were stored at –20°C until determination.

Determination methods: Plasma glucose concentration was determined by a hexokinase enzyme method using a dry-slide technology (Vet Test, Idexx Laboratories Inc., Tokyo, Japan). Plasma insulin concentration was determined by a sandwich ELISA (Feline Insulin Measurement Kit, Morinaga Institute of Biological Science Inc., Yokohama, Japan) [15, 26].

Statistics: Differences were evaluated by multiple comparison test using Scheffe's F test (Statcel-The Useful Addin Forms on Excel. 2nd ed, OMS publishing, Tokyo, Japan). A P value below 0.05 was considered statistically significant.

RESULTS

Figure 1 shows plasma glucose and insulin concentrations after injection of normal saline and 50% glucose solution. In saline, plasma glucose concentrations did not change after injection. Plasma insulin concentrations increased slightly to 1.0 or 1.2 *n*g/m*l* from 4 to 20 min after injection in 2 cats (Nos. 1 and 2), and did not alter in another cat. After injection of glucose, plasma glucose concentrations increased rapidly, then decreased gradually with time. Plasma glucose concentrations remained at higher levels of 166 to 229 mg/d*l* even 60 min after injection. Plasma insulin concentrations increased gradually to 2.4 to 3.7 *n*g/m*l* until 45 or 60 min after injection.

Figure 2 indicates plasma glucose and insulin responses after injection of arginin, alanine, leucine, fat emulsion solutions, valine, methionine, taurine and glutamine. After injection of arginin, plasma glucose concentrations were within the normal ranges. Plasma insulin concentrations elevated rapidly to higher levels at 2.7 to 7.3 *n*g/m*l* until 4 min after injection, then fell to the basal levels 20 min after injection in all five cats. After injection of alanine and leucine, plasma glucose concentrations were within the normal ranges. Plasma insulin concentrations increased mildly and at a slow rate. Also in fat emulsion, plasma glucose concentrations were within the normal ranges, and increases in plasma insulin concentrations were very small. In valine,

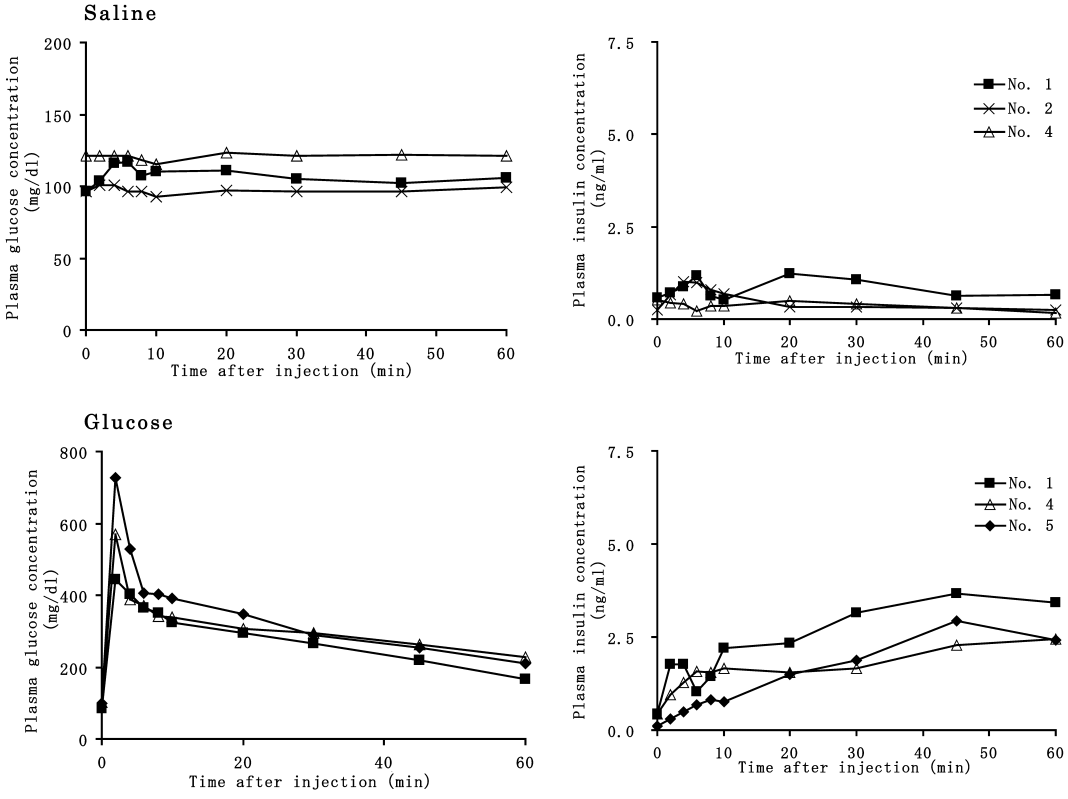


Fig. 1. Plasma glucose and insulin concentrations after injection of saline and glucose.

methionine, taurine and glutamine, plasma glucose concentrations were below 150 mg/d*l* after injections. Temporary mild increases in insulin secretion were observed in one or two cats after injections of valine (No. 1), methionine (No. 4), taurine (No. 1), and glutamine (Nos. 1 and 5).

Table 1 shows basal plasma insulin concentration (I_0), insulin peak response (IPR), insulin peak time (IPT) and area under the curve (AUC) of plasma insulin concentrations. The I_0 levels were almost the same in all substances. The IPR was considerably high in glucose and arginine, and mildly higher in alanine and leucine. The IPT was shorter in arginine than in glucose, alanine, leucine and fat emulsion. The AUC of plasma insulin concentration from 0 to 10 (AUC_{0-10}) was larger in arginine than normal saline ($P<0.01$) and glucose ($P<0.05$). The AUC from 0 to 20 min (AUC_{0-20}) was larger in arginine than normal saline ($P<0.05$). The AUC from 0 to 60 min (AUC_{0-60}) was larger in glucose than normal saline ($P<0.01$), because of the later increase in plasma insulin concentration. Among amino acids and fatty acids, AUC_{0-60} mildly enlarged in arginine, leucine, alanine, and fat emulsion, but these evidenced no significant difference. The AUCs in valine, methionine, taurine and glutamine were similar to that in saline.

DISCUSSION

Temporary mild increases in insulin secretion were observed after injection of normal saline, valine, methionine, taurine and glutamine in the present study. In cats, it is well known that stress elicits insulin secretion [20]. Alterations in plasma insulin concentrations may be induced by the stress of retention and needle puncture. Thus, the alterations may indicate no insulin responses after injection of these substances.

A representative example of insulin release is thought to be a mechanism of insulin release against glucose in human as follows [2, 9]: glucose enters the pancreatic β-cells through the glucose transporter 2. Then, glucose is transduced to glucose-6-phosphate (G6P) by the rate-controlling enzyme, glucokinase. Adenosine triphosphate (ATP) is produced from G6P by the glycolytic system. In mitochondrias, ATP is also produced by the tricarboxylic acid cycle. ATP/ADP rate elevates according to the increase in intracellular ATP concentrations. Next, the ATP-sensitive K^+ channel (KATP channel) closed, thereby inducing depolarization of the cell membrane. On depolarization, the voltage-dependent Ca^{++} channels (VDCC) open, and Ca^{++} flows into the β-cells. Rapid increase of intracellular Ca^{++} concentration extricates insulin secretory granules into extracellular fluid. In the cat, the deficit of glucokinase involves a longer glucose clearance time.

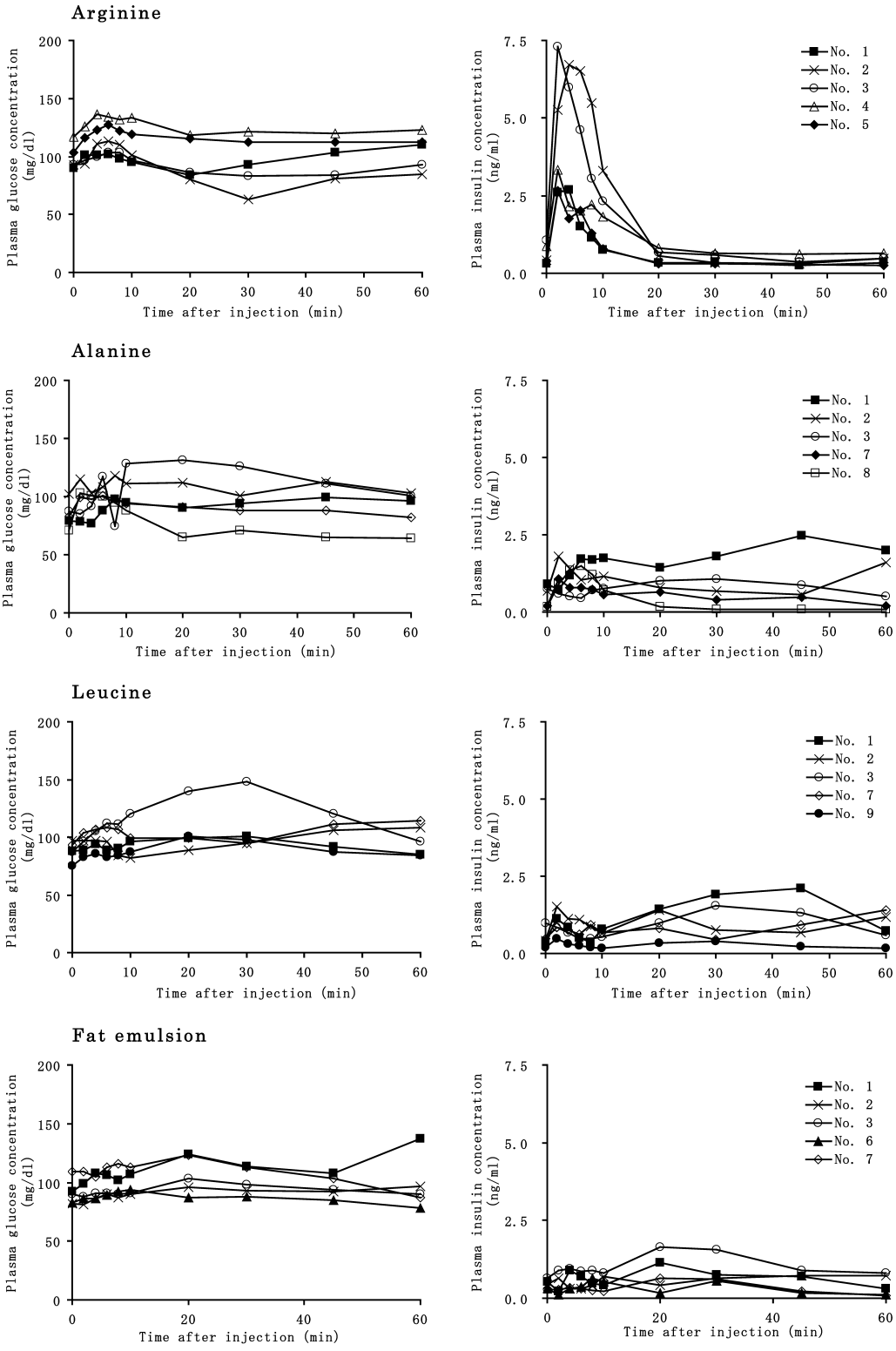
In the present study, insulin secretions increased significantly after injection of arginine, and mildly after injection of alanine, leucine and fat mixture emulsion. The effect of arginine in insulin secretion differs from that of glucose. The mechanism of insulin stimulation by arginine was thought to depolarize the β-cell membrane, because of its transport in the cell in a positively-charged form [4]. The depolarization in turn activates VDCC, and Ca^{++} influx ensues. Therefore, arginine was thought to be able to secrete a large amount of insulin in a short period of time. The mechanism of insulin secretion by alanine is probably due to the cotransport of alanine with Na^+ in the pancreatic β-cells [19]. The small depolarization of cell membranes augments slightly the Ca^{++} influx, while the increase in intracellular Na^+ influences Ca^{++} handling [10]. Leucine largely enters the β-cells through a Na^+-independent transport system, and is well metabolized. According to the depolarization of cell membranes, Ca^{++} flowed into the β-cells [10]. Moreover, the insulin-releasing action of fatty acids reportedly depends more on the esterification or the oxidation of fatty acids [14, 25], though the precise mechanism has been unclear.

In cats, insulin secretions were stimulated after injection of some amino acids and fatty acids. There is a possibility that a large amount of alanine or leucine secretes more insulin. However, solubilities of alanine and leucine in water are 15.8 g/100 m*l* and 2.38 g/100 m*l* at 20, much lower than that of glucose (91 g/100 m*l* at 25°C). Consequently, the injection of these amino acids in a higher dose in a short time is

Table 1. Insulin responses to intravenous amino acid and fatty acid administrations in cats

Substance	n	I_0 (*n*g/m*l*)	IPR (*n*g/m*l*)	IPT (min)	AUC_{0-10} (*n*g/ml/10 min)	AUC_{0-20} (*n*g/ml/20 min)	AUC_{0-60} (*n*g/m*l*/60 min)
Saline	3	0.4 ± 0.2	0.8 ± 0.5	–	6.5 ± 2.4	12.5 ± 4.3	32.5 ± 15.9
Glucose	3	0.3 ± 0.2	3.0 ± 0.7	50.0 ± 8.7	11.0 ± 4.9	27.7 ± 10.5	129.7 ± 34.3[a)]
Arginine	5	0.6 ± 0.3	4.2 ± 2.0[a)]	2.8 ± 1.1	30.5 ± 16.7[a,c)]	42.2 ± 22.4[b)]	59.2 ± 24.7
Alanine	5	0.5 ± 0.3	1.5 ± 0.6	17.0 ± 19.5	10.0 ± 3.1	18.9 ± 6.7	52.9 ± 35.5
Leucine	5	0.5 ± 0.3	1.4 ± 0.6	27.8 ± 25.8	6.8 ± 2.6	14.5 ± 5.8	53.9 ± 25.2
Fat emulsion	5	0.4 ± 0.1	0.9 ± 0.4	22.6 ± 13.6	5.0 ± 2.2	11.7 ± 5.7	37.1 ± 19.7
Valine	5	0.7 ± 0.4	0.8 ± 0.3	–	4.8 ± 1.7	9.8 ± 3.3	32.8 ± 13.3
Methionine	5	0.4 ± 0.2	0.8 ± 0.5	–	4.8 ± 1.8	9.1 ± 3.3	25.1 ± 8.5
Taurine	5	0.5 ± 0.4	0.8 ± 0.6	–	4.9 ± 2.7	9.0 ± 4.8	24.4 ± 16.1
Glutamine	5	0.4 ± 0.3	0.8 ± 0.3	–	3.1 ± 1.2	7.5 ± 2.5	26.5 ± 9.9

Data are expressed as mean ± SD; n: number of cats; I_0: basal plasma insulin concentration; IPR: insulin peak response; IPT: insulin peak time; hyphen indicates no clear peak time. AUC: area under the curve of insulin. a) and b) indicates a significant difference from the data of saline ($P<0.01$ and $P<0.05$, respectively). c) indicates a significant difference from the data of glucose ($P<0.05$).


Arginine
Plasma glucose concentration (mg/dl)
Time after injection (min)
Plasma insulin concentration (ng/ml)
No. 1
No. 2
No. 3
No. 4
No. 5
Alanine
No. 7
No. 8
Leucine
No. 9
Fat emulsion
No. 6

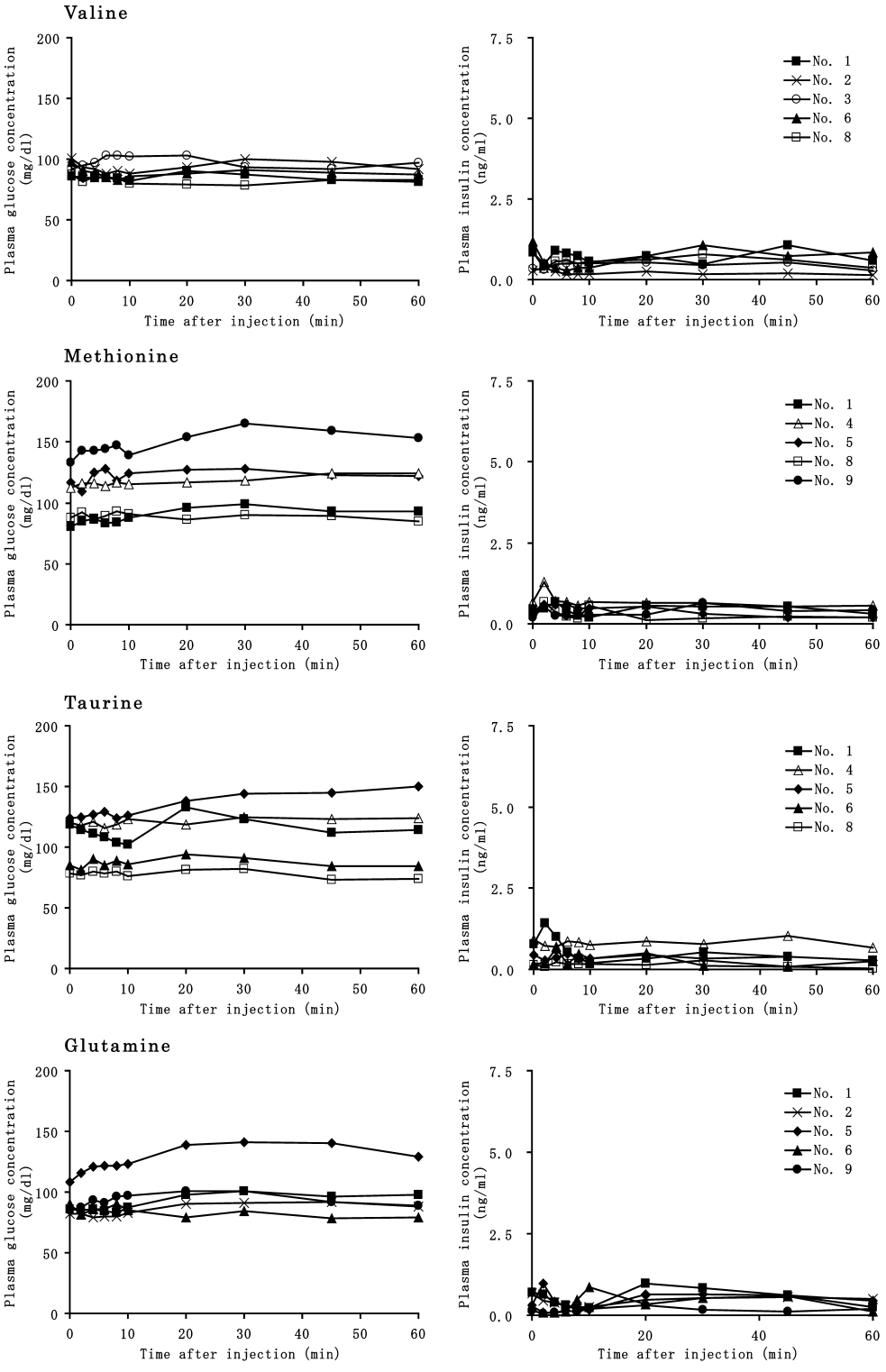


Fig. 2. Plasma glucose and insulin concentrations after injection of arginine, alanine, leucine, fat emulsion, valine, methionine, taurine and glutamine.

not possible.

Among arginine, alanine, leucine and fatty acids, the insulinotropic effect of arginine was strongest. The effects of alanine, leucine and fatty acids were low and differed in individuals. Although we have not tested insulin responses by all amino acids, we consider that arginine is a useful substance for clinical application in cats, because the arginine injection does not induce hyperglycemia, the testing time was short (20 min) from the concentration curve, and the 10% solution (Arginine Injection, Ajinomoto Pharma. Co., Ltd., Tokyo, Japan) is available in the human medical market for the growth hormone stimulation test in Japan [23].

Insulin secretions were stimulated after injection of arginine, alanine, leucine and fatty acids, however, the effects of alanine, leucine and fatty acids were mild and differed in individuals. Arginine has the strongest insulinotropic effect among these substances. Although additional research is warranted on diseased cats, intravenous arginine tolerance tests may be useful clinically for estimation of insulin responses in cats.

REFERENCES

1. Appleton, D. J., Rand, J. S., Priest, J. and Sunvold, G. D. 2001. Determination of reference values for glucose tolerance, insulin tolerance, and insulin sensitivity tests in clinically normal cats. *Am. J. Vet. Res.* **62**: 630–636.
2. Ashcroft, F. M. and Ashcroft, S. J. H. 1992. Mechanism of insulin secretion. pp. 97–150. *In*: Insulin Molecular Biology to Pathology (Ashcroft, F. M. and Ashcroft, S. J. H. eds.), IRL press, Oxford.
3. Biourge, V., Nelson, R. W., Feldman, E. C., Willits, N. H., Morris, J. G. and Rogers, Q. R. 1997. Effect of weight gain and subsequent weight loss on glucose tolerance and insulin response in healthy cats. *J. Vet. Intern. Med.* **11**: 86–91.
4. Charles, S., Tamagawa, T. and Henquin, J. C. 1982. A single mechanism for the stimulation of insulin release and $^{86}Rb^{+}$ efflux from rat islets by cationic amino acids. *Biochem. J.* **208**: 301–308.
5. Curry, D. L., Morris, J. G., Rogers, Q. R. and Stern, J. S. 1982. Dynamics of insulin and glucagon secretion by the isolated perfused cat pancreas. *Comp. Biochem. Physiol. A.* **72**: 333–338.
6. Ettinger, S. J. 2005. The physical examination of the dog and cat. pp. 2–9. *In*: The Textbook of Veterinary Internal Medicine, 6th ed. (Ettinger, S. J. and Feldman, S. C. eds.), Elsevier Saunders Inc., St. Louis.
7. Fasching, P., Ratheiser, K., Nowotny, P., Uurzemann, S., Parker, S. and Waldhaus, W. 1994. Insulin production following intravenous glucose, arginine, and valine: different pattern in patients with impaired glucose tolerance and non-insulin-dependent diabetes mellitus. *Metabolism* **43**: 385–389.
8. Hellman, B., Sehlin, J. and Taljedal, I.-B. 1971. Effects of glucose and other modifiers of insulin release on the oxidative metabolism of amino acids in micro-dissected pancreatic islets. *Biochem. J.* **123**: 513–521.
9. Henquin, J. C. 1994. Cell biology of insulin secretion. pp. 56–80. *In*: Joslin's Diabetes Mellitus, 13th ed. (Kahn, C. R., Weir G. C., eds.), Lea & Febiger, Philadelphia.
10. Henquin, J. C. and Meissner, H. P. 1986. Cyclic adenosine monophosphate differently affects the response of mouse pancreatic β-cells to various amino acids. *J. Physiol.* **381**: 77–93.
11. Kaneko, J. J., Mattheeuws, D., Rottiers, R. P. and Vermeulen, A. 1977. Glucose tolerance and insulin response in diabetes mellitus of dogs. *J. Small Anim. Pract.* **18**: 85–94.
12. Kitamura, T., Yasuda, J. and Hashimoto, A. 1999. Acute insulin response to intravenous arginine in nonobese healthy cats. *J. Vet. Intern. Med.* **13**: 549–556.
13. Lerner, R. L. and Porte, D. 1971. Relationships between intervenous glucose loads, insulin responses and glucose disappearance rate. *J. Clin. Endocrinol.* **33**: 409–417.
14. Malaisse, W. J., Malaisse-Lagae, F., Sener, A. and Hellerstrom, C. 1985. Participation of endogenous fatty acids in the secretory activity of the pancreatic β-cell. *Biochem. J.* **227**: 995–1002.
15. Nakaya, M., Kito, Y., Matsuki, N., Shibata, H., Touhata, Y., Tamahara, S. and Ono, K. 2009. A novel sandwich enzyme-linked immunosorbent assay for feline insulin, *J. Vet. Med. Sci.* **71**: 1005–1007.
16. Nelson, R. W., Himsel, C. A., Feldman, E. C. and Bottoms, G. D. 1990. Glucose tolerance and insulin response in normal-weight and obese cats. *Am. J. Vet. Res.* **51**: 1357–1362.
17. O'Brien, T. D., Hayden, D. W., Johnson, K. H. and Stevens, J. B. 1985. High dose intravenous glucose tolerance test and serum insulin and glucagon levels in diabetic and non-diabetic cats: relationships to insular amyloidosis. *Vet. Pathol.* **22**: 250–261.
18. Palmer, J. P., Walter, R. M. and Ensinck, J. W. 1975. Arginine-stimulated acute phase of insulin and glucagon secretion I. In normal man. *Diabetes* **24**: 735–740.
19. Prentki, M. and Renold, A. E. 1983. Neutral amino acid transport in isolated rat pancreatic islets. *J. Biol. Chem.* **258**: 14239–14244.
20. Rand, J. S., Kinnaird, E., Baglioni, A., Blackshaw, J. and Priest, J. 2002. Acute stress hyperglycemia in cats is associated with struggling and increased concentrations of lactate and norepinephrine. *J. Vet. Intern. Med.* **16**: 123–132.
21. Reaven, G. M. and Olefsky, J. M. 1974. Relationships between insulin response during the intravenous glucose tolerance test, rate of fractional glucose removal and the degree of insulin resistance in normal adults. *Diabetes* **23**: 454–459.
22. Rottiers, R., Mattheeuws, D., Kaneko, J. J. and Vermeulen, A. 1981. Glucose uptake and insulin secretory responses to intravenous glucose loads in the dog. *J. Vet. Res.* **42**: 155–158.
23. Russell, M., Bredella, M., Tsai, P., Mendes, N., Miller, K. K., Klibanski, A. and Misra, M. 2009. Relative growth hormone deficiency and cortisol excess are associated with increased cardiovascular risk markers in obese adolescent girls. *J. Clin. Endocrinol. Metab.* **94**: 2864–2871.
24. Takasu, M., Kishima, Y., Nishii, N., Ohba, Y., Maeda, S., Miyazaki, K., Murase, T. and Kitagawa, H. 2007. Insulin and glucagon secretory patterns during propionate and arginine tolerance tests in Japanese black cattle with growth retardation. *J. Vet. Med. Sci.* **69**: 69–72.
25. Tamarit-Rodriguez, J., Vara, E. and Tamarit, J. 1984. Starvation-induced changes of palmitate metabolism and insulin secretion in isolated rat islets stimulated by glucose. *Biochem. J.* **221**: 317–324.
26. Yasuda, K., Nishii, N., Takashima, S., Takagi, M., Touhata, Y., Shibata, H. and Kitagawa, H. 2010. Clinical evalution of an enzyme-linked immunosorbent assay kit for determination of plasma insulin concentrations in cats. *Jpn. J. Vet. Med. Assoc.* **63**: 449–452 (in Japanese).