Effects of Anesthesia on Metabolic Responses to Mixed Meal Tolerance Test in Cynomolgus Monkeys

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Abstract

Mixed meal tolerance test (MMTT) is commonly used in diabetic research in experimental animal models and humans to evaluate glucose metabolic regulation by entero-insular axis. Since gastric intestinal physiology is affected by anesthesia, it could in turn affect the MMTT responses. The aim of the present study was to evaluate the impact of sedation on MMTT responses in normal (n=7, Control), pre-diabetic (n=6, Pre-DM), and diabetic (n=7) cynomolgus macaques based on their metabolic profiles including fasting glucose and insulin as well as ivGTT responses. Following oral gavage of a standard liquid mixed meal, both the hyperglycemic and insulinogenic responses were greater in the conscious monkeys than sedated ones in the control, but not in DM monkeys, while in Pre-DM monkeys, only the hyperglycemic, but not insulinogenic responses were higher in conscious than sedated condition. Thus, the present data demonstrate for the first time in nonhuman primates (NHP) that anesthesia blunted the normal physiology responses to MMTT, while such responses were already diminished in DM NHPs.

Introduction

Elevated blood glucose stimulates pancreas secretion of insulin and inhibits its glucagon release, which is regulated by entero-insular axis, a complex neural hormonal response. Intravenous (iv) administration of glucose, such as iv glucose tolerate test (ivGTT), graded glucose infusion test (GGI), hyperglycemic clamp, etc, is the most direct way to activate glucose-stimulated insulin release from β-cells, which, however, may not effectively involve entero-insular axis since many incretin hormones, such as GLP-1, GIP, etc. secrete from the gut with ingestion of glucose and other nutrients, such as proteins and lipids, release into the circulation, then reach to their targets, such as beta and alpha cells to regulate insulin and glucagon release. Therefore, oGTT or MMTT gives a more comprehensive assessment not only for the functions of beta and alpha cells, but also for the entero-insular axis, such as GLP-1 and other incretin signaling. For the laboratory experiments in animal models, especially in nonhuman primate (NHP), to avoid the impact of complicated procedure-induced stress on the experimental outcomes, many studies, such as GTTs, GGI, clamps, etc. are often undertaken with anesthesia. However, it has been known that anesthesia would significantly slow gut emptying, impact absorption of glucose and other nutrients and affect secretion of gut hormones at various degrees in different animal models. Thus, it may also affect the responses to the tests. The present study aimed to investigate the impact of anesthesia on metabolic responses to MMTT in normal and diabetic cynomulgus macaques (Macaca fascicularis).

Methods

Animals:

Adult non-insulin-treated cynomolgus macaques (*Macaca fascicularis,* n=20*)* of either gender were used in the study. The experimental procedures were approved by the Institutional Animal Care and Use Committee (IACUC) and were performed in accordance with the Guide for the Care and Use of Laboratory Animals of the United States National Institutes of Health (NIH).

Intravenous glucose tolerance test（ivGTT):

-Animal state: Overnight fasting

-Anesthesia:10mg/kg ketamine was administered intramuscularly initially, additional 5~10 mg/kg ketamine was supplied if needed.

-Glucose solution: 50% dextrose solution, i.v.

-Dose: 0.25g/kg .

-Blood collection: ~ 1mL blood was at 0 (pre-dosing), 3, 5, 7, 10, 15, 20 and 30 min post-dosing for serum glucose and insulin assays.

-Area under the curve (AUC) was calculated using the trapezoid method.

Mixed Meal Tolerance Test (MMTT)

-Animal state: Overnight fasting

-Sedation: ketamine was dosed IM to achieve initial sedation at 10 mg/kg and continuous sedation by supplemental IM injections of ketamine at 5-10 mg/kg when necessary.

-Meal Composition : The mixed meal consists of Ensure Plus with additional sucrose (24 g per 8 fl oz). The meal was orally administrated via a nasogastric tube at a volume of 5 ml/kg.

-Blood collection: Blood samples were collected at (fasting) -10, -3, 10, 20, 30, 60, 90, 120 and180 minutes for blood biochemistry and hormones analysis.

Data analysis

Data are presented as mean ± SEM. The results from different groups were compared using ANOVA comparisons. Statistical significance was set at $p < 0.05$.

Table 1. Basic characterizations (* p < 0.05,Compared to Normal group)

	Normal (N=7)	Pre-DM (N=6)	DM (N=7)
Age (yr)	6.73 ± 0.25	15.54 ± 1.17*	17.94 ± 0.97*
Weight (kg)	7.24 ± 0.63	7.88 ± 0.84	7.46 ± 0.61
Height (cm)	80.4 ± 1.19	79.0 ± 2.30	73.1 ± 2.00*
Waist Circumferences (cm)	38.8 ± 2.27	45.2 ± 2.31	48.4 ± 2.28*
Hip Circumference (cm)	36.8 ± 1.95	41.3 ± 1.57	40.3 ± 1.93
BMI (kg/m^2)	11.44 ± 0.75	12.05 ± 0.63	13.64 ± 0.90*

Results

Fig. 1. Effects of anesthesia on glucose response to MMTT.

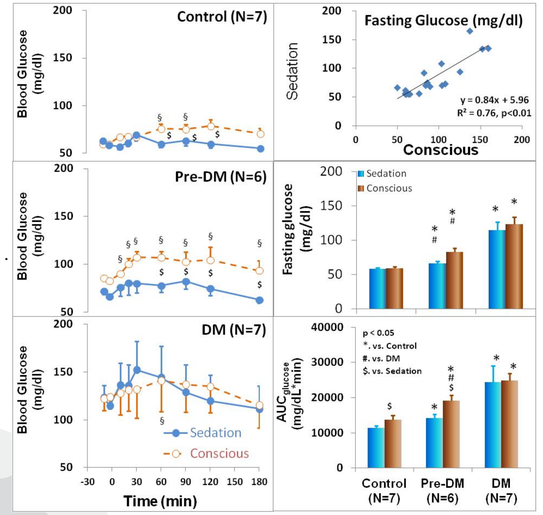

Fig. 2. Effects of anesthesia on insulin response to MMTT.

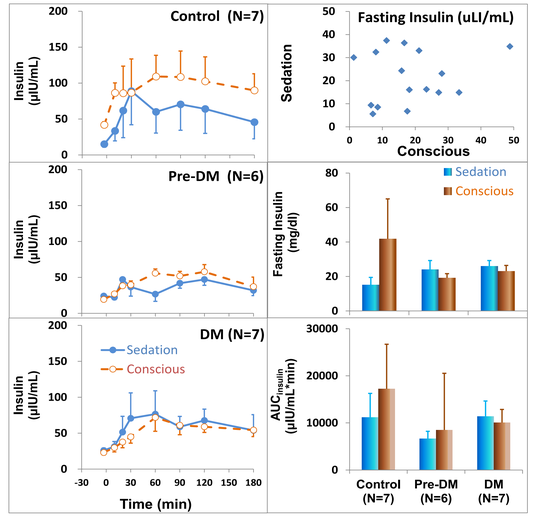

Table 2. Effects of anesthesia on hormonal responses to MMTT

Area Under the Curve (pg/mL*180 min)	Control (n=3)		Diabetes (n=3)	
	Sedation	Conscious	Sedation	Conscious
Glucagon	1,598,806 ± 226,915	1,554,342 ± 127,787	1,550,861 ± 102,562	1,986,779 ± 326,220
GLP-1	8,318 ± 2,745	8,147 ± 2,132	6,921 ± 1,623	5,651 ± 1,308
GIP	16,651 ± 5,797	56,918 ± 15,399	27,545 ± 12,609	73,297 ± 27,953
Ghrelin	592,921 ± 421,331	225,209 ± 14,209	265,704 ± 117,299	170,866 ± 64,116
Leptin	146,872 ± 8,530	188,867 ± 10,365 *	163,011 ± 12,334	169,346 ± 16,198
Resistin	197,963 ± 22,471	364,156 ± 35,362	271,083 ± 54,871	312,135 ± 83,331
Visfatin	85,160 ± 36,975	342,210 ± 147,907	201,367 ± 74,392	415,647 ± 202,882
IL-6	7,988 ± 2,955	35,940 ± 9,062	7,760 ± 518	24,375 ± 4,985 *
MCP-1	85,160 ± 36,975	342,210 ± 147,907	201,367 ± 74,392	415,647 ± 202,882
PAI-1	162,108 ± 7,698	299,749 ± 5,644	246,747 ± 31,054	282,971 ± 75,763

*** p < 0.05, Compared with sedation.**

Summary

1. **The fasting glucose level was higher in the Pre-DM and the highest in the DM groups compared to the control group, which was not significantly affected by anesthesia.**
2. **The hyperglycemic responses to MMTT measured by AUC were significantly greater in the conscious than sedated condition in both control and Pre-DM, but not in DM group.**
3. **The MMTT-induced insulin increase was enhanced, although not statistically significant, in the conscious compared to sedated condition only in the control, but not in Pre-DM and DM groups.**
4. **Most hormonal responses were similar with or without anesthesia, but leptin and IL-6 significantly increased in conscious controls and DMs.**
5. **The data demonstrate that anesthesia may reduce MMTT response, probably via attenuation of the intestinal absorption and other physiological responses at different levels in pre-diabetic and diabetic stages.**