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ORIGINAL ARTICLE

Influence of physical restraint on the onset of experimentally induced diabetes mellitus

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The role of intermittent repeated physical restraint on the onset of diabetes mellitus (DM) was investigated in this study. The study compared the onset of DM in mice dosed with streptozotocin (STZ), a DM-inducing drug, with immediate subsequent exposure to either physical restraint stress or non- exposure to the stress. Sixty mice were randomly assigned to 6 equal groups: 0 mg kg $^{-1}$ STZ with no stress, 0 mg kg^{-1} STZ with stress, 25 mg kg^{-1} STZ with no stress, 25 mg kg^{-1} STZ with stress, 50 mg kg^{-1} STZ with no stress, 50 mg kg^{-1} STZ with stress. Blood glucose, body weight and food consumption were regularly determined during the study. On day18, mice were killed and blood for corticosterone determination was collected. Increase in STZ dosage or physical restraint stress lowered bodyweight on days 4-18 (P<0.05). Increasing STZ dosage elevated the blood glucose on day7-18 (P<0.05). Restraint lowered blood glucose on day 11-18 (P<0.05). Interaction between both factors was significant on day11-18 (P<0.05). Nine out of 10 of the 50 mg kg^{-1} STZ no-stress mice and 2 out of 10 of the 50 mg kg^{-1} STZ stress mice developed DM. Physical restraint was a more important predictor for whether a mouse would have been diabetic or not. Physical restraint delayed the onset of diabetes mellitus.

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INTRODUCTION

Diabetes is a huge global health issue and is expected to continue growing. Currently, almost 10% of the world's adult population has diabetes (WHO, 2013). Diabetes mellitus has been defined as a metabolic disorder of multiple origins characterized by chronic hyperglycemia with disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action or both (Alberti and Zimmet, 1998). The well-known classifications of diabetes, type 1 and type 2 (Castano and Eisenbarth, 1990, Alberti and Zimmet, 1998) ensure patients are classified based on pathogenesis rather than treatment. Diabetes type 1 is a result of the destruction of the beta cells of the pancreatic islets whereas diabetes type 2 is characterized by the malfunction of insulin action or secretion, either of which may be more prevalent, but both are usually seen at the time of diagnosis (Castano and Eisenbarth, 1990, Roep, 2008). In general, the development of diabetes involves several disease-causing processes, including processes which destroy the beta cells of the pancreatic islets, causing insulin deficiency, and others that cause resistance to the function of insulin (Alberti and Zimmet, 1998). However, much remains to be learned about diabetes' pathogenesis and relationships with environmental factors, such as physical exertion and other forms of stress.

Stress is the body's non-specific response to real or perceived threats/demands (Selye, 1976). It may be categorized into two types: acute and chronic. During acute stress the hormones cortisol and adrenaline are released, causing increased blood pressure and heart rate and heightened immune system and memory, which can be helpful for a short period of

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time. The response to chronic stress, however, can be detrimental. Blood pressure, heart rate, appetite, bodyweight, cholesterol, triglyceride, and blood sugar levels have been shown to change during chronic stress. These are not only risk factors for heart disease, atherosclerosis, stroke and obesity, but also diabetes (McEwen, 2008). For example psychological stress including physical restraint stress has been shown to enhance the production of immunosuppressive cytokines linked to an increased occurrence of infectious disease, which demonstrates that the immunosuppressive actions of stress do in fact translate into significant adverse health effects (Curtin *et al*., 2009).

Cortisol levels are known to rise in response to stress. Because the main effect of cortisol involves raising blood glucose levels, we therefore hypothesized, in the present study, that stress including physical restraint stress (that normally involves physical exertion) would accelerate the onset of chemically-induced diabetes mellitus in otherwise normal healthy Swiss ICR mice.

MATERIALS AND METHODS

Experimental procedure

Sixty hsd:ICR(CD-1®)_mice were randomly assigned to 6 groups of 10 mice each. Two factors, physical restraint stress at 2 levels and streptozotozin at 3 levels (dosages were in mg kg^{-1} bodyweight), were arranged in a factorial manner to produce 6 treatments: 0 mg kg^{-1} STZ with no stress (0 mg kg^{-1} STZ no-stress), 0 mg kg^{-1} STZ with stress (0 mg kg^{-1} STZ stress), 25 mg kg^{-1} STZ with no stress (25 mg kg^{-1} STZ no-stress), 25 mg kg^{-1} STZ with stress (25 mg kg^{-1} STZ stress), 50 mg kg^{-1} STZ with no stress (50 mg kg^{-1} STZ no-stress), 50 mg kg^{-1} STZ with stress (50 mg kg^{-1} STZ stress). Mice were housed individually and allowed 3 days to acclimatize before experimentation.

Mice received once-a-day intraperitoneal injections of the assigned dose of a diabetes-inducing chemical, streptozotocin (STZ) in citrate buffer or citrate buffer alone for the first three days of the study. Streptozotocin-treated mice are commonly used to model diabetes type 1 due to the ability of streptozotocin (STZ) to destroy the insulin-producing beta cells of the pancreatic islets of Langerhans (Reagan *et al*., 1999, Maiese *et al*., 2007). Baseline animal weight, feed weight, and glucose levels were taken on day 0 for all mice and STZ or buffer injections were given.

Non-fasting glucose measurements were taken three times a week beginning on day 0 for both groups using blood glucose meters (Freestyle Freedom Lite, Catalog number 70914, NDC 99073-0709-14, Distributed by Abbott Diabetes Care Inc) and appropriate test strips (Freestyle Lite Blood Glucose Test Strips). Mice were weighed twice each week. Feed weight was taken two times per week. Feed was refilled and reweighed as needed to calculate food consumption. Mice exposed to physical restraint stress as described by Bonneau *et al*., (1993) were placed in 50 ml well-ventilated tubes packed lightly with approved nesting material for 6 hours beginning at approximately the same time each day during which time the control mice were free in their cages but without access to feed and water. After the 6 hour-period the stressed mice were returned to their cages and both the stressed and the control mice were again given access to feed and water. The study lasted for 18 days. Such exposure to physical restraint stress for 6 hours per day for 18 days was considered chronic stress. This was in line with the study of Gao *et al.,* (2006) who considered physical restraint stress for 6 hours per day for 21 days to be chronic stress while one time 6-hour restraint was considered acute stress. At the end of the 18 days of the study, mice were euthanized and blood was collected for serum preparation. The animal protocol was approved by the University Committee on Animal Care (UCAC) at East Tennessee State University, Johnson City, TN, a AALAC accredited research institution.

Corticosterone Assay

The corticosterone assay was performed according to a colorimetric competitive enzyme immunoassay method outlined by the manufacturer of a commercial kit (Assay Designs© Corticosterone Enzyme Immunoassay Kit, Ann Arbor, MI, Catalog No. 900 -097). Standards, blanks, samples and reagents were

placed in the designated wells as per the kit's instructions. The plates were then covered, incubated at room temperature and thereafter contents were decanted. The wells were then washed and conjugate solution added to the total activity wells before stopping the reaction in all wells. The extent of colorimetric change in each well was read using a microplate reader (Benchmark Microplate Reader, Bio-Rad, Hercules, CA) at a wavelength of 405 nm with a correction between 570 and 590 nm.

Statistical Analysis

The SAS statistical software (version 9.2, SAS Institute Inc. 2002, Cary, NC) was used to perform statistical analyses (SAS, 2002). Since there were two factors: STZ and stress, a two-way ANOVA (analysis of variance) was used. The study was a factorial one in which the response is observed at all factor-level combinations of the independent variables. Two-way ANOVA model splits the total variability into four sources of variability, which in this case are: the main effects of STZ, the main effects of stress, the possible interaction between STZ and stress, and the unexplained variability from all sources not accounted for by the main effects and interaction, known as error (Ott *et al*., 2001). In order to compare changes among individuals, baseline values were taken. Baseline measurements were also used to ensure that the independence assumption of ANOVA model was not violated by checking for randomization of the measurements (DeVeaux *et al*., 2008). ANCOVA (analysis of covariance) was used to account for the effect of baseline measurement values when analyzing the values for any one subsequent specific time (Wildt and Ahtola, 1978). Baseline values were used as covariates when studying the difference in values between treatments in subsequent times. In this manner, one could determine if the baseline level was an important factor.

Data sets of the repeated measures nature (with multiple measurements of a response variable on the same experimental unit) were analyzed using repeated measures. Repeated measures analysis was performed on bodyweight, glucose and food consumption and the Akaike information criterion (AIC) was used to select the method to be used for correlation. Repeated measures analysis was done to investigate whether there were significant differences within groups at the different times when the data were collected and also between the different treatment groups. Longitudinal analysis (Fitzmaurice *et al*., 2008) was applied to blood glucose, body weight and cumulative food consumption data and response profiles for the measurements taken at different times were examined to characterize the patterns of change in the respective response variable over time in the different groups.

The two factors (STZ and physical restraint stress) were evaluated using logistic regression procedure in SAS for their suitability in predicting diabetes development (persistent blood glucose level of at least 200 mg dl^{-1}, (Padmanabhan *et al*., 2006) in the mice. Differences were considered significant at $P<0.05$.

RESULTS

In general, statistical analyses revealed that STZ, physical restraint stress, and their interaction became statistically significant in causing the measurable differences in the various response (dependent) variables among treatment groups. When repeated measures analysis was performed on bodyweight, glucose and food consumption, the interaction between stress and STZ was dependent on the day of the measurement (Tables 1, 2 and 3).

Blood Glucose

Persistent non-fasting blood glucose level of at least 200 mg dl^{-1} was considered as evidence of the mice having developed diabetes mellitus in this study (Padmanabhan *et al*., 2006). There were no significant differences between the baseline blood glucose values among the different treatment groups ($P>0.05$, Table 1). However by day 7 of the study, the differences, using ANOVA, among mice dosed with different levels of STZ became significant ($P<0.013$), although there was no difference as yet between stressed and non-stressed mice (Table 1). STZ becoming significant meant that different groups of mice no longer had the same glucose levels. The 50 mg kg^{-1} STZ mice showed significantly higher blood glucose level than the lower

STZ doses (0 and 25 mg kg^{-1}). STZ was also significant on day 7 using ANCOVA with glucose day 0 as a covariate. The difference was clearer ($P<0.0052$) when using ANCOVA with glucose day 0 as a covariate because baseline variability was negated. The effect of STZ on blood glucose level remained significant for the rest of the experimental days with the higher STZ dose mice showing significantly higher blood glucose compared to the lower STZ dose mice (Table 1). The mice that received 0 or 25 mg kg^{-1} STZ showed no difference in mean blood glucose between the stressed and non-stressed mice (Table 1).

The effect of physical restraint stress on blood glucose became significant from day 11 and then on day 16 until the end of the experiment on day 18. Physical restraint stress on these days lowered the blood glucose levels (Table 1). By day 9 of the experimental period, there was a mild interaction between the effect of STZ and physical restraint stress on blood glucose level ($P<0.0609$, Table 1). An interaction occurs when the effects of one factor change for the different levels of another factor. By day 11, the STZ-stress interaction was significant ($P<0.0029$) and remained significant ($P<0.05$) for the rest of the experimental period. Interaction between STZ and physical restraint stress was noted at the highest STZ dose (50 mg kg^{-1}) group and it was this group that had a much higher mean for glucose change from the baseline values. STZ affected the blood glucose of mice differently depending on whether they were stressed or not. Surprisingly it was the stressed mice that showed lower levels of blood glucose compared to the non-stressed mice (Table 1). Nine out of 10 of the 50 mg kg^{-1} STZ no-stress mice and 2 out of 10 of the 50 mg kg^{-1} STZ stress mice developed diabetes mellitus (persistent blood glucose of ≥200 mg dl^{-1}). Hyperglycemia in these mice was observed as early as day 4 and con-

Table 1: Blood glucose level (means, mgdl^{-1}) in mice dosed different levels of STZ and then subsequently either stressed or not stressed for 18 days (n=10)

STZ	Stress	Day 0	Day 2	Day 4	Day 7	Day 9	Day 11	Day 14	Day 16	Day 18
					Simple effect means					
0	No	126	100	142	121	121	114	97	111	118
	Yes	121	107	143	126	126	121	122	112	138
25	No	128	123	132	132	120	135	118	114	139
	Yes	132	111	118	117	120	124	120	127	118
50	No	123	105	163	168	210	262	265	288	346
	Yes	120	112	135	146	148	162	154	183	191
						Main effect means				
0		122	104	143	123	124	118	110	111	128
25		130	117	125	125	115	129	119	120	128
50		122	109	149	157	179	212	210	236	269
	No	125	110	146	140	147	170	160	171	201
	Yes	124	110	132	130	131	136	132	141	149
Source of Variation					***Probability***					
STZ		0.3626	0.2769	0.2179	0.0052	0.0006	0.0001	0.0001	0.0001	0.0001
Stress		0.9445	0.9098	0.2309	0.2549	0.2575	0.0097	0.0954	0.0419	0.0014
STZ * Stress		0.8476	0.4119	0.5739	0.4765	0.0609	0.0029	0.0026	0.003	0.0001
SD[1]		22	26	43	35	53	50	63	57	60

[1] ***SD = Pooled standard deviation***

tinued for the rest of the study period.

Unstructured correlation was used for the repeated measures analysis because it yielded a lower value for the Akaike information criterion (AIC). The effects of STZ, stress, day and interactions STZ-stress, STZ -day, stress-day and STZ-stress-day on blood glucose level were all significant. Similar to the ANOVA results, the repeated measures model showed that STZ, physical restraint stress, day and their interactions were significant factors in causing differences in blood glucose among the six treatment groups. Group response profiles for mean blood glucose levels showed that while the mean glucose levels for the 0 and 25 mg kg^{-1} STZ stress and no-stress groups remained relatively stable, it was evident that the blood glucose levels of the 50 mg kg^{-1} STZ no-stress group increased throughout the study (Figure 1) whilst the glucose levels of the 50 mg kg^{-1} STZ stress group increased only slightly. Profiles for all other groups except the 50 mg kg^{-1} STZ no-stress seemed to be parallel to each other implying that blood glucose levels in these groups changed in the same manner across time, regardless of the treatment group.

When the two factors (STZ and physical restraint stress) were evaluated for their suitability in predicting diabetes development in the mice, restraint was significant ($P<0.0130$). The predicted probabilities were 98.1% concordant. This means that the model was 98.1% correct in its prediction of all possible concordant pairs of mice that developed diabetes with those that did not develop diabetes.

Bodyweight

Baseline bodyweight values were not significantly different among all the treatment groups ($P>0.005$), a confirmation of the randomness of the assignment of the mice to the 6 treatment groups (Table 2). By day 4 of the experiment, the effects of STZ and physical restraint stress were significantly affecting bodyweight ($P<0.05$, Table 2). This remained so for the rest of the experimentation period. Higher levels of both factors significantly reduced the body weight of the mice. The highest

Table 2: Bodyweight (means, g) in mice dosed different levels of STZ and then subsequently either stressed or not stressed for 18 days (n=10).

STZ	Stress	Day 0	Day 4	Day 9	Day 11	Day 15	Day 18
				Simple effect means			
0	No	32	33	34	34	34	36
	Yes	32	31	31	31	32	33
25	No	32	33	34	34	34	35
	Yes	32	31	31	31	32	33
50	No	32	31	32	32	32	33
	Yes	32	30	30	30	31	32
				Main effect means			
0		32	32	32	33	33	34
25		32	32	32	33	33	34
50		32	31	31	31	31	32
	No	32	32	33	33	33	35
	Yes	32	31	31	31	32	32
Source of Variation				***Probability***			
STZ		0.5582	0.0148	0.0386	0.0253	0.0093	0.0121
Stress		0.587	0.0003	0.0001	0.0001	0.0003	0.0001
STZ * Stress		0.7308	0.8681	0.6315	0.703	0.5603	0.5412
SD[1]		1.6	1.5	1.8	1.8	1.9	1.9

[1] SD = Pooled standard deviation

STZ dosed animals weighed significantly less (P<0.05) compared to either the 0 or 25 mg kg^{-1} STZ dosed animals. Also the mice exposed to physical restraint stress weighed significantly less (P<0.05) compared to non-stressed animals. There were no significant interaction effects of STZ and physical restraint stress on bodyweight.

Unstructured correlation was used for the repeated measures analysis because it yielded a lower value for the Akaike information criterion (AIC). Repeated measures analysis of body weight data showed that STZ, physical restraint stress, day and stress-day interaction had significant effects on bodyweight. Like the ANOVA results, the repeated measures analysis showed that STZ and physical restraint stress were significant factors in causing the differences in body weight among the six treatment groups. Group response profiles for the mean body weights showed that all treatment groups lost weight in the beginning before starting to gain weight with exceptions in the 0 and 25 mg kg^{-1} STZ no-stress groups (Figure 2). The 50 mg kg^{-1} STZ no -stress group lost the most weight and remained the lightest group in the study. There was very little variability existing among the treatment groups on day 0 compared with day 18. The higher dose of STZ caused a decrease in bodyweight as did stress.

Food consumption

There were no significant effects of STZ or physical restraint stress on cumulative food consumption (Table 3). Repeated measures analysis on food consumption however showed that the effect of day on cumulative food consumption was significant (P<0.05). None of the other factors had a significant effect on food consumption. Differences from day-to-day were however expected because meas-

Table 3: Cumulative food consumption (mean, g) in mice dosed different levels of STZ and then subsequently either stressed or not stressed for 18 days (n=10).

STZ, mg kg^{-1}	Stress	Day 0	Day 8	Day11	Day15	Day18
			Simple effect means			
0	No	0	60	87	116	139
	Yes	0	51	73	99	118
25	No	0	54	71	96	113
	Yes	0	51	69	95	112
50	No	0	67	91	121	142
	Yes	0	51	69	94	112
			Main effect means			
0		0	56	80	108	128
25		0	52	70	95	112
50		0	59	80	107	127
	No	0	60	83	111	131
	Yes	0	51	70	96	114
Source of Variation			***Probability***			
STZ			0.5914	0.4956	0.4619	0.4222
Stress			0.0682	0.0935	0.1145	0.1254
STZ * Stress			0.5651	0.5773	0.5188	0.5421
SD[1]			20	30	35	42

[1] SD = Pooled standard deviation

urements were cumulative in nature. The group response profiles for cumulative feed consumption showed that the profiles remained parallel to each other with more variability among groups being observed on day 18 than at the beginning, thus giving an indication that the feed consumption increased similarly for all groups.

Corticosterone

The two-way ANOVA analysis on serum corticosterone levels at the end of the study indicated that STZ and physical restraint stress and their interaction had significant effects on corticosterone levels (with *P* values of <0.0048, <0.0001, and 0.0010, respectively). Simple effect means of serum corticosterone levels (pg ml^{-1}) for 0 mg kg^{-1} STZ no-stress, 0 mg kg^{-1} STZ stress, 25 mg kg^{-1} STZ no-stress, 25 mg kg^{-1} STZ stress, 50 mg kg^{-1} STZ no-stress, 50 mg kg^{-1} STZ stress were 1375, 1278, 400, 3064, 884, 6164, respectively. Main effect means of serum corticosterone levels (pg ml^{-1}) for the different levels of STZ (0, 25 and 50 mg kg^{-1} bodyweight) were 1327, 1732 and 3524, respectively and those of no-stress and stress treatments were 886 and 3502, respectively. Increasing the STZ levels had an effect of raising the serum corticosterone level with stressed mice showing higher serum corticosterone levels.

DISCUSSION

The drug streptozotocin (STZ) used in this study destroys the beta cells of the pancreatic islets and it

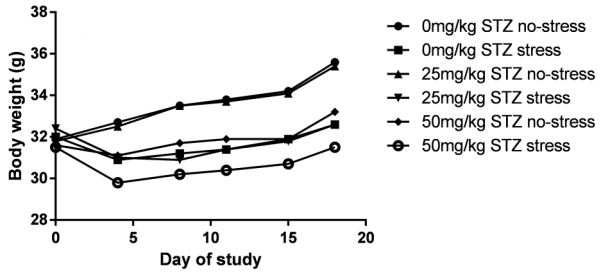

Figure 1: Response profile for blood glucose level (means, mg dl^{-1}) in mice dosed different levels of STZ (mg kg^{-1} bodyweight) and then subsequently either stressed or not stressed for 18 days (n=10).

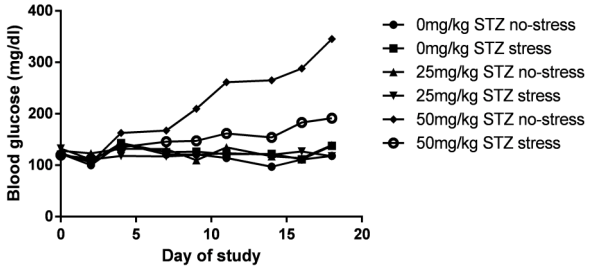

Figure 2: Response profile for bodyweight (means, g) in mice dosed different levels of STZ (mg kg^{-1} bodyweight) and then subsequently either stressed or not stressed for 18 days (n=10).

is commonly used to induce diabetes mellitus type 1 in study animals. Low doses of streptozotozin have been shown to produce diabetes mellitus. For example, a low-dose STZ regimen of 50 mg kg^{-1} injected intraperitoneally for 5 consecutive days in fasted mice produces hyperglycemia within 2 weeks (Breyer *et al.*, 2005).

In some studies it has been suggested that the incidence of diabetes may vary depending upon environmental factors such as stress (Fitzpatrick *et al.*, 1992). In the study by Fitzpatrick *et al.,* (1992), serum glucocorticoid concentrations in basal and stress conditions were measured in non-obese diabetic mice and C57BL/6 control mice. It was found that the diabetic mice generally exhibited a higher corticosterone response than the controls (Fitzpatrick *et al.*, 1992). In the present study, STZ was found to be the source of the observed difference in corticosterone levels among the different groups. These observations are in agreement with previous findings where STZ-induced diabetes was accompanied by elevated levels of serum corticosterone (Mizuno *et al.*, 1999, Oishi *et al.*, 2004). Another study that also observed high resting levels of plasma corticosterone in diabetic rats concluded that those observations suggested that diabetic rats were in a chronic stress condition (De Nicola *et al.*, 1977).

Interestingly, in this study the non-stressed 25 mg kg^{-1} and 50 mg kg^{-1} STZ mice had higher glucose

levels but it was the stressed 25 mg kg^{-1} and 50 mg kg^{-1} STZ mice that had higher corticosterone levels. Many studies have researched the effects of stress on diabetic mice and rats (Huang *et al.*, 1981, Meehan *et al.*, 1987, Fitzpatrick *et al.*, 1992, Korolkiewicz *et al.*, 1999, Reagan *et al.*, 1999, Bates *et al.*, 2007, Bazhan *et al.*, 2007). Reagan *et al.,* (1999) examined the neurological changes induced by 7 days of physical restraint stress in STZ diabetic rats and found that the hippocampus of diabetic rats was extremely susceptible to stress. This research group reported that diabetic rats showed dendritic atrophy of pyramidal neurons, increased GLUT3 mRNA and protein expression in the hippocampus and stress additionally caused an increase of receptors for insulin-like growth factor (IGF) in the hippocampus (Reagan *et al.*, 1999).

In a study by Korolkiewicz *et al.,* (1999), using rats made diabetic by a single 70 mg kg^{-1} STZ injection 5 weeks prior to the study showed that stressful stimuli such as food deprivation and cold challenge contributed to the elevated susceptibility of diabetic gastric mucosal damage. Bazhan and others (Bazhan *et al.*, 2007) found that repeated light emotional stress decreased the development of obesity and diabetes type 2 in mice with Agouti yellow mutation which produces an obese diabetic phenotype (Bazhan *et al.*, 2007). Using borderline, overt, or severe diabetic mice induced by STZ, Meehan *et al.*, (1987) studied glycemic responses of mice to the stress of a resident -intruder encounter and stress of blood drawing from the retro-orbital sinus. They found that plasma glucose elevation in overtly and severely diabetic mice is not as specific to behavior as in non-diabetic mice (Meehan *et al.*, 1987). Bates *et al.,* (2007) found that intermittent restraint delayed hyperglycemia and improved glucose control in Zucker diabetic fatty rats.

In many of the above studies stress was commonly applied to already diabetic animals while in the present study low levels of STZ were administered and physical restraint stress was immediately applied to mice even before exhibiting diabetic symptoms. Baseline values for glucose and body weight obtained in this study were not significantly different from each other. This was an important foundation as it meant that there was no bias among treatment groups before treatments began. The effect of STZ on blood glucose levels became significant by day 7 of the experimental period. This observation is in agreement with the findings of Mizuno *et al.*, (1999) who noted that, one week after STZ injection, they could define mice as diabetic when they exhibited plasma glucose greater than 300 mg dl^{-1}.

Surprisingly, by day 9 and the subsequent days, the 50 mg kg^{-1} STZ stressed mice exhibited lower levels of glucose than their non-stressed counterparts. This finding is contrary to expectations of the effects of stress on blood glucose. Stress is normally accompanied with a rise in glucocorticoids with a resultant rise in blood glucose, a condition that would be expected to favour the development of diabetes mellitus. There have been similar findings of reduced hyperglycemia in certain types of stress such as suspension by nape of neck stress (Kosovskii *et al.*, 1988), intermittent restraint (Bates *et al.*, 2007), and light repeated physical restraint stress (Bazhan *et al.*, 2007).

Research by Kosovskii *et al.,* (1988) comparing types of stress and the development of diabetic syndrome found that mice stressed through cavitary operation exhibited the signs of diabetes while those stressed through suspension by nape of neck did not. They suggested that the differences could be attributed to the fact that cavitary operation resulted in limited mobility while mice stressed by suspension had increased movement while trying to escape. Reduced hyperglycemia in mice stressed by suspension by nape of neck could provide one possible explanation for the reduced hyperglycemia in restraint-stressed mice in the present study: while the mice were being stressed by physically restraining them, they were working escape, which was a form of exercise and this seemed to have reduced the occurrence of high blood glucose levels in these mice. Further support for a role of physical restraint in reducing or delaying hyperglycemia is suggested by the hampering of development of diabetes type 2 by repeated physical restraint stress (Bazhan *et al.,* 2007) and also by the delayed hyper-

glycemia and improved glucose control by intermittent restraint (Bates *et al*., 2007).

In the above studies (Kosovskii *et al*., 1988, Bates *et al*., 2007, Bazhan *et al*., 2007) stress was applied to animals genetically predisposed to diabetes. In the present study, however, stress was applied to normal healthy Swiss ICR mice not genetically predisposed to diabetes. Even in these animals where diabetes mellitus could develop due to chemical exposure, the presence of physical restraint stress (that involves physical exertion of the animals) slowed down the development of consistent hyperglycemia associated with diabetes mellitus. The findings in the present study are supported by observations of Huang and others (Huang *et al*., 1981) who stressed STZ-injected mice (60 mg kg^{-1} body weight) through shock stimulation and found that none of the mice developed diabetes mellitus type 1 if they were stimulated an hour after STZ injection whereas non-stimulated mice developed hyperglycemia and became diabetic within 6 and 8 weeks after STZ injection. Indeed it would seem to suggest that stress that causes physical exertion may mitigate development of hyperglycemia.

From this study, STZ, stress and their interaction were significant factors in causing the differences in blood glucose levels among the six treatment groups. Group response profiles for glucose showed that the 50 mg kg^{-1} STZ non-stress group had the greatest increase in glucose levels suggesting that physical restraint stress, in some way, protected the mice from development of hyperglycemia. Group response profiles for body weight showed that physical restraint stress seemed to have made mice in all groups to lose weight initially with the exception of 0 mg kg^{-1} STZ and 25 mg kg^{-1} STZ non-stress groups respectively. This finding is in agreement with that of Reagan and others (Reagan *et al*., 1999) who observed that body weight significantly decreases as the diabetic state develops in STZ-injected mice. It was interesting to note that even though there were no significant differences in the cumulative amount of feed consumed, there were significant differences in bodyweight among the different groups. Because the higher bodyweight was in non-stressed mice, it may be that physical restraint stress may have led to a higher energy consumption compared to the stressed animals thus resulting in body weight differences.

Serum corticosterone levels were affected by both STZ and stress levels with stressed mice showing higher corticosterone levels. Mice on higher STZ levels showed elevated serum corticosterone levels when they were stressed perhaps indicating that STZ may in some way be predisposing the mice to easier elevation of corticosterone when mice are stressed. Logistic regression analysis showed that physical restraint stress was a significant factor in the prediction of the development of diabetes (hyperglycemia of over 200 mg dl^{-1}). The physical struggle (exertion) of the mice during restraint may have mitigated the development of diabetes in stressed mice.

CONCLUSION

The present study suggests that restraint stress that tends to lead to increased physical exertion may attenuate the onset of diabetes mellitus type 1 in mice. Physical restraint was predictive for whether a mouse would be diabetic or not. It seemed to mitigate the development of diabetes mellitus (persistent hyperglycemia) in mice and hence delayed the onset of diabetes mellitus.

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COMPETING INTERESTS

The authors declare that they have no competing interests.

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