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Acute stress-induced changes in hormone and lipid levels in mouse plasma

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ABSTRACT: We evaluated the conventional acute stress model in mice and investigated the stress-induced changes in concentrations of plasma lipids and hormones such as corticosterone (CORT), insulin, glucagon, triiodothyronine (T_3), and thyroxine (T_4). Stress induction for 120 min using tape-immobilisation and restraint resulted in an increase in the plasma levels of CORT, insulin, glucose, total cholesterol (TC), and low density lipoprotein-cholesterol compared with unstressed mice. Stress also resulted in a decrease in the concentrations of T_3, T_4, triglycerides (TG), and high density lipoprotein-cholesterol. However, the amount of glucagon did not change. Moreover, the concentrations of T_3, T_4, TC, TG, and lipoprotein cholesterol were maintained at constant levels over the recovery periods after stress induction; however, CORT, glucose, and insulin concentrations decreased as a function of time. Statistical correlations between the parameters that changed in response to acute stress were also investigated. In contrast, stress induction for 30 or 60 min did not cause substantial changes in the lipid profiles, although there were fluctuations in the levels of some hormones. This study thus introduces an appropriate model for the study of the acute stress response of lipids and hormones and our data suggest that acute stress affects the levels of plasma lipids, especially cholesterol, in mice.

Keywords: acute stress; corticosterone; hormones; plasma lipids; metabolism; mice

Glucocorticoids, which are released from the adrenal cortex, are well-known vertebrate stress response hormones. Stress-induced glucocorticoids elicit many physiological changes that enable animals to react appropriately to stress conditions. For example, glucocorticoids increase blood glucose levels (Leung and Munck 1975) and stimulate hydrolysis of triglycerides in adipocytes (Gregoire et al. 1991), which have important roles in energy balance and supply in animals.

Corticosterone (CORT) is the main glucocorticoid found in many vertebrates, including species of rodents, birds, and reptiles, and cortisol is the primary glucocorticoid in humans. CORT, a steroid hormone produced in the adrenal cortex, is induced by acute and chronic stress in mice, and both types of stress increase the plasma concentration of CORT (Barlow et al. 1975). CORT has many physiological functions involving energy metabolism, immune reactions (Cyr et al. 2007), and even memory (Dominguez et al. 2014). It is also related to plasma lipids, especially cholesterol, in rats. Stress such as lever press escape/avoidance was described to induce an increase in plasma cholesterol levels and a decrease in aortic cholesterol levels, respectively; these phenomena were accompanied by an elevation of CORT (Starzec et al. 1983). However, acute and chronic stresses cause different physiological changes: for example, plasma metabolites and the responses to chronic stress (e.g., rat corticotropin responses to long-term exposure to cold temperatures such as 5 °C) were dependent on model species and stress-induction methods (Dallman and Bhatnagar 2001). Acute stress is also a controversial

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subject, and results are influenced by the species of target animal, stress-duration time, kind of stressor, and other experimental conditions (Sutanto and de Kloet 1994). Moreover, with regard to lipid distribution and metabolism, the acute and long-term effects of glucocorticoids within tissues and tested models remain unclear and controversial (Peckett et al. 2011).

In this study, we evaluated the acute stress model in mice using tape-immobilisation and restraint stressors. We also investigated acute stress-induced changes in CORT concentrations and plasma lipid profiles, as well as the correlations between these changes to clarify the effect of acute stress on lipid metabolism. In addition to CORT, concentrations of other hormones such as insulin, glucagon, and thyroid hormones were measured to evaluate the relationship between the hormonal and lipid changes in plasma because these hormones modulate energy homeostasis, which is closely related to the levels of plasma lipids.

MATERIAL AND METHODS

Animals. Male C57BL/6 mice were obtained from Samtako Bio Korea (Osan, South Korea). All mice (eight weeks old, 18–20 g) were used after one week of quarantine and acclimation. The mice were housed five per cage in a temperature- and lighting-controlled room (23 ± 1 °C; alternating 12 h periods of light and dark). Mice were fed a restricted calorie Laboratory Rodent Diet 5001 diet (approximately 13 kcal per mouse per day; LabDiet; St. Louis, MO, USA) during all experimental periods because of the close relationship between plasma levels of lipid including hormones and calorie intake. The level of calorie intake was ensured by using only the mice that had consumed the allocated diet completely. The experimental design was approved by the committee for the Care and Use of Laboratory Animals at Chonnam National University (CNU IACUC-YB-2014-22).

Stress induction. Stress was induced in mice using tape-immobilisation and restraint methods as described (Sanchez et al. 2002). Briefly, mice were subjected to tape-immobilisation stress as follows: mouse tails were fixed with adhesive tape to a table in a supine position for 30, 60, or 120 min, and the mice were allowed to recover for the indicated periods under normal housing conditions. After each recovery time, the mice were immediately sacrificed for the measurements of plasma lipid and hormone concentrations. For the induction of restraint stress, mice were introduced into a small custom-made acryl cylinder (restrainer) with open head and tail gates. The mice were then almost immobilised in the restrainer for 30, 60, or 120 min. During the recovery periods after stress induction, mice were not fed but had free access to water. Mice (n = 10) not exposed to either stress were used as controls.

Sampling. Mice were anaesthetised using sodium pentobarbital (50 mg/kg) before sacrifice. Blood was taken from the inferior vena cava and collected into two tubes, one of which contained potassium EDTA to measure the plasma concentration of triglycerides (TG), total cholesterol (TC), and lipoprotein cholesterols, such as high density lipoprotein-cholesterol (HDL-C) and low density lipoprotein-cholesterol (LDL-C). The other tubes were heparinised for analysis of the plasma concentrations of CORT, insulin, glucagon, triiodothyronine (T_3), and thyroxine (T_4). Blood plasma was obtained by centrifugation at 4 °C.

Assays. The plasma was shipped on ice to Seoul Clinical Laboratories (Seoul, South Korea), where analysis was completed within 72 h after collection. Glucose concentrations were measured using a glucose oxidase reagent set (Pointe Scientific; Canton, MI, USA). TC levels was measured using a Pureauto S assay kit (JW Pharmaceutical; Seoul, South Korea). HDL-C and TG levels were measured using an ADVIA 2400 (Siemens; Washington, DC, USA). LDL-C concentrations were calculated using the modified Friedewald equation (McAuley et al. 2001). Plasma CORT was analysed using high-performance liquid chromatography-fluorimetry (Mason et al. 1992). Insulin concentrations were measured using the ADVIA CentaurÔ Insulin Lite Reagent and Solid Phase (Siemens), and detected using the ADVIA CentaurÔ XP (Siemens). CORT concentrations were assayed using the VetTest chemistry analyser (IDEXX; Westbrook, ME, USA). Total and free concentrations of T_3 and T_4 were assayed using a radioimmunoassay kit and reagents (Quest Diagnostics; Madison, NJ, USA).

Statistical analysis. Statistical analyses were performed using two-way ANOVA in SPSS version 19.0 (SPSS Inc.; Chicago, IL, USA). For comparisons of data in Figures 1, 2, and 3, $P < 0.05$ was considered as statistically significant. Correlations between

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Table 1. Hormone and lipid concentrations in the plasma of normal mice

CORT	Insulin	Glucagon	Total T_3	Total T_4
15.2 ± 3.3 (μg/l)	1.8 ± 0.4 (ng/ml)	98 ± 16 (pg/ml)	783 ± 142 (ng/l)	35.1 ± 5.2 (μg/l)
Glucose	TG	TC	HDL-C	LDL-C
1.18 ± 0.20 (g/l)	2.5 ± 0.4 (mmol/l)	7.7 ± 0.9 (mmol/l)	4.2 ± 0.6 (mmol/l)	1.3 ± 0.2 (mmol/l)

Data are presented as mean ± SEM (n = 10)

plasma lipid profiles (or glucose) and CORT as well as other hormones were examined using Pearson correlation analysis in an online-calculator (www.socscistatistics.com/tests/pearson).

RESULTS

Table 1 summarises the concentrations of hormones and plasma lipids in the normal (unstressed) control mouse group. To investigate acute stress-induced changes in hormones, including lipid profiles in plasma and the relationship between these levels, we employed conventional methods for acute stress induction in rodents in a stress duration- and recovery time-dependent manner. When mice were subjected to tape-immobilisation stress for 30 min, plasma CORT level at 1 h after the stress termination was enhanced by about 300% compared with that of unstressed mice (Figure 1A). However, the hormone concentration gradually decreased during the subsequent recovery period and approached that of normal conditions at about 12 h after the stress. Figure 1A also shows that TG and TC levels in plasma marginally changed after stress or over the indicated recovery time. Similar results were obtained using tape-immobilisation for 60 min; an increase in CORT level by 400–500% after 1 h of recovery was observed (Figure 1B). Conversely, glucose values substantially increased after the stress but then decreased over time. Restraint stress for 30 or 60 min did not cause changes in plasma lipid concentrations, although CORT release was enhanced by approximately 250–400% (results not shown). In neither experiment, however (stress-

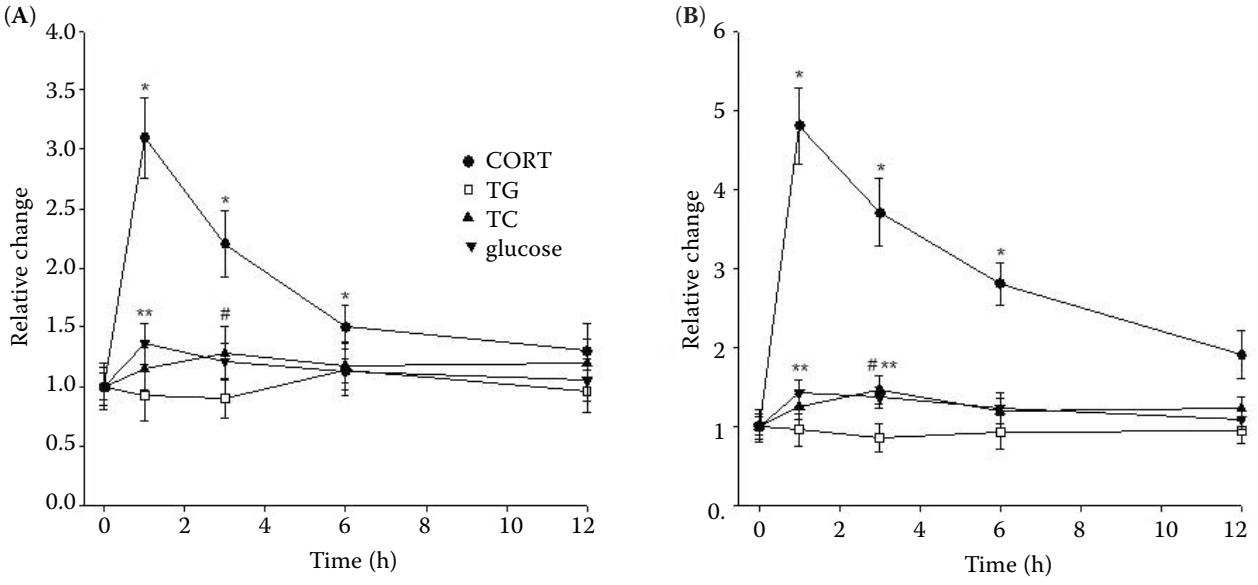

Figure 1. Changes in CORT and lipid levels in plasma during recovery after tape-immobilisation stress. Mice were subjected to stress for 30 min (**A**) or 60 min (**B**); then, the concentrations of CORT (●, *), TG (□), TC (▲, #), and glucose (▼, **) were measured at each indicated recovery time (1, 3, 6, or 12 h) after stress termination. Time zero (0 h) represents the parameters of unstressed mice as a control; these values were set to one (1). The y-axis represents the relative changes in the measured parameters compared to the control value. Results are mean ± SE of 10 animals (n = 10) per each group. Significant differences compared with time zero (control) values are shown by *, #, **$P < 0.05$

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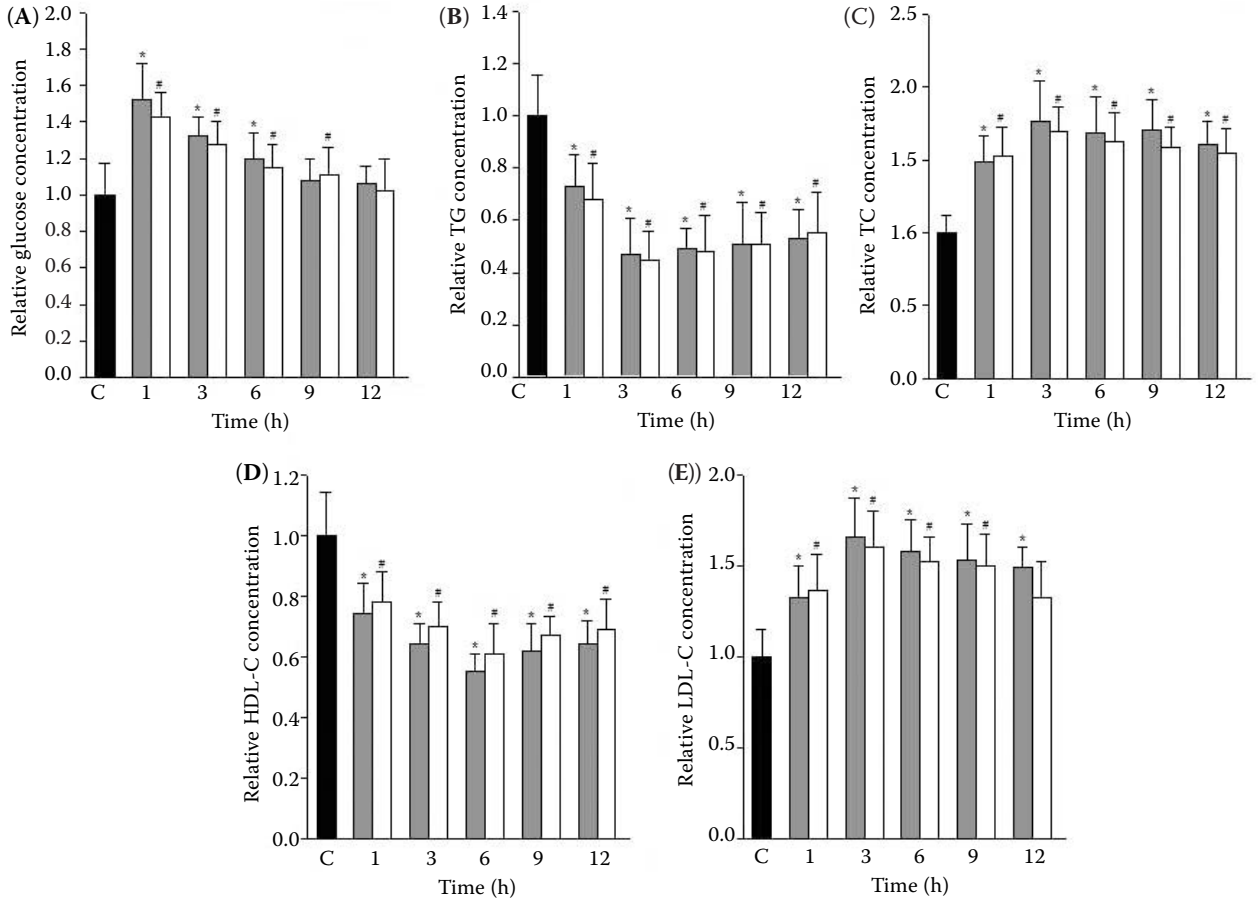

Figure 2. Recovery time-dependent changes in glucose (**A**), TG (**B**), TC (**C**), HDL-C (**D**), and LDL-C (**E**) after stress induction for 120 min using tape-immobilisation (gray bars) and restraint (open bars) methods. Black bars (control) represent plasma levels of each parameter that were measured in unstressed mice. Control values were set to one (1) and the data are presented as a relative scale. The indicated numbers on the *x*-axis represent each recovery time after stress termination. Results are mean ± SE of 10 animals (n = 10) per each group. Significant differences compared with the control values are shown by *, $^{\#}P < 0.05$

duration for 30 or 60 min), were the lipid and hormones levels determined immediately after exposure to stress.

In contrast with the results in Figures 1 and 2 shows that the concentrations of plasma lipids and glucose were significantly altered during the recovery periods after tape immobilisation or restraint stress induction for 120 min: TG levels were maximally reduced to 0.47–0.54, which was dependent on the recovery time, when the lipid values of the control (normal) group were set to one. Glucose and TC levels were increased by approximately 150% and 180%, respectively. Figure 2 also shows that the acute stressors decreased HDL-C concentrations to approximately 0.55–0.78 and increased LDL-C values by 150–170%.

Figure 3 shows the hormonal changes in plasma during recovery periods after stress induction for 120 min. Both stressors stimulated the release of CORT and insulin by 600–700% and 170–200%, respectively; this was also dependent on the recovery time. The values gradually decreased over the recovery time, which is similar to the pattern seen in Figure 1. T_3 and T_4 concentrations were also measured, and these values were maximally reduced by about 50% compared with those of the unstressed group after stress termination. In contrast with the results for CORT and insulin, the decreased T_3 and T_4 levels were maintained and showed little changes for at least 12 h.

To investigate the relationships between parameters affected by acute stress, Pearson correlation coefficients (r) were calculated. However, because the parameters under investigation fluctuated during the recovery periods, maximum negative or positive values were presented after the calcu-

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Table 2. Pearson correlation coefficients (*r*) between the parameters in plasma following acute stress induction

Stress	Tape-immobilization							
	glucose	TG	TC	HDL-C	LDL-C	insulin	T_3	T_4
CORT	0.326	–0.256	0.457	–0.532	0.424	0.338	–0.084	–0.115
Insulin	0.146	–0.213	0.248	–0.109	0.122	–	–0.102	–0.079
T_3	–0.155	0.288	–0.158	0.132	–0.077	–0.064	–	–
T_4	–0.212	0.245	–0.161	0.058	–0.063	–0.051	–	–
	Restraint							
CORT	0.314	–0.273	0.433	–0.584	0.452	0.375	–0.124	–0.109
Insulin	0.122	–0.189	0.235	–0.084	0.131	–	–0.074	–0.083
T_3	–0.209	0.213	–0.113	0.071	–0.062	–0.051	–	–
T_4	–0.223	0.236	–0.138	0.083	–0.054	–0.069	–	–

lations of the values with each indicated recovery time. In addition, the correlation degrees were defined as 'strong', 'moderate', and 'weak' states, for which each corresponding *r* value was in the range of 0.5–1.0 (or –1.0 to –0.5), 0.3–0.5 (or –0.5 to –0.3), and 0.1–0.3 (or –0.3 to –0.1), respectively.

Table 2 shows that CORT levels were strongly correlated with HDL-C (–0.532 and –0.584 in response to immobilisation and restraint stress, respectively). This result indicates that CORT levels are directly related to cholesterol metabolism. The CORT levels were also moderately correlated with TC, glucose, and insulin concentrations in both acute stress conditions, which supports an effect of CORT on the regulation of these parameters. However, the correlations were weak with T_3 and T_4; *r* values were calculated to be –0.084 and –0.124 (–0.115 and –0.109 for T4) in response to immobilisation and restraint stress, respectively. Insulin was also correlated with the stress-induced concentration changes in glucose, TG, TC, and LDL-C although the correlations were weak. In the cases of T_3 and T_4, glucose, TG, and TC were weakly correlated with these hormones while other plasma parameters showed no discernible relationship.

DISCUSSION

Despite the fact that the stressors used in this study are the most commonly used models for acute stress induction, these methods have been applied in a wide range of different ways to analyse the effects of stress on physiological changes in rodents (Pare and Giavin 1986). Moreover, the methods often yield contradictory results in the analyses. Therefore, we re-evaluated experimental conditions for acute stressors (tape-immobilisation and restraint) that affect plasma lipids and hormones. To undertake the present study, we first measured the concentrations of selected hormones and plasma lipids in a normal (unstressed) mouse group (Table 1). While the values showed significant individual differences, overall levels were consistent with previous results (Malisch et al. 2007; Fuchsl et al. 2013).

Similar results to the current data have been reported previously: a stress-responsive increase in glucose and CORT levels as well as a decrease in TG concentrations was described in a species of bird (Remage-Healey and Romero 2001). However, contradictory results have also shown that serum lipid levels in rats were not related to the stress intensity (Armario et al. 1986). Nonetheless, the results depicted in Figures 1 and 2 indicate that stress induction for over 60 min should be employed for acute stress models in order to study the effects of stress on lipid profiles in mouse plasma.

In addition to the validation of acute stressors, the present study provides systematic results for stress-induced changes in plasma parameters of mice. In summary, it was concluded from the results of Figure 2 that both acute stresses induced increases in glucose, TC, and LDL-C, and decreases in TG and HDL-C. It should be also noted that, when the data in Figure 2 are analysed in more detail, the results show a certain regular pattern: TC and LDL-C levels were enhanced as TG and HDL-C levels decreased after stress-induction, and the altered values were maintained for the indicated recovery periods. However, glucose levels recovered over time and approached those of normal

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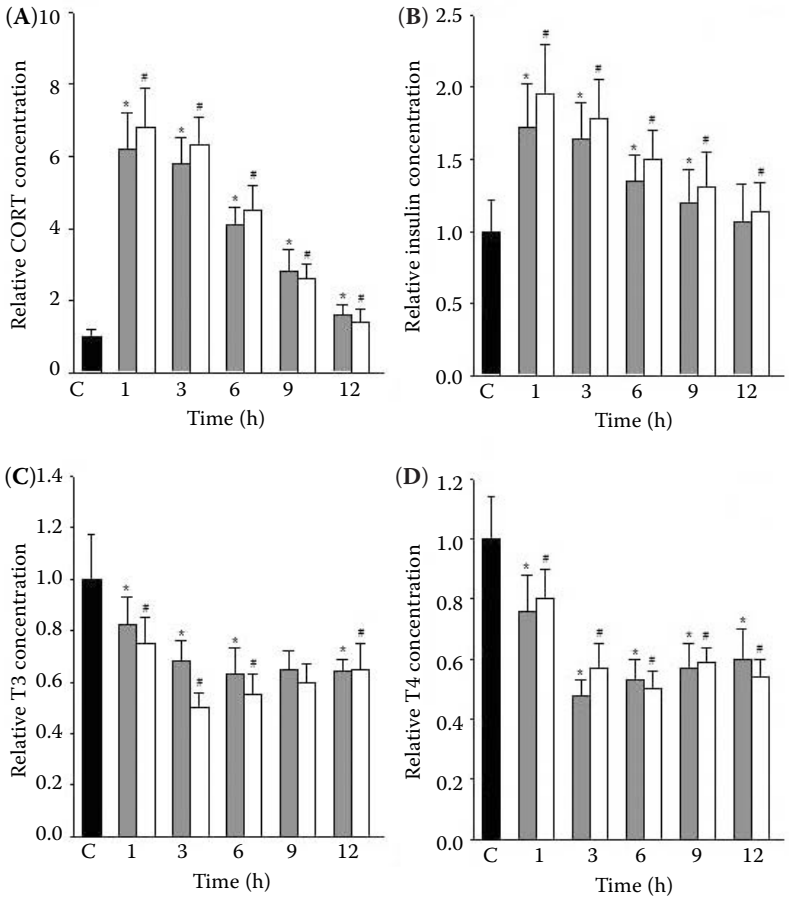

Figure 3. Recovery time-dependent changes in CORT (**A**), insulin (**B**), T_3 (**C**), and T_4 (**D**), after stress induction for 120 min using tape-immobilisation (gray bars) and restraint (open bars). Black bars represent plasma levels of each parameter that were measured in unstressed mice as a control. Control values were set to one (1) and the data are presented as a relative scale. The indicated numbers on the *x*-axis represent each recovery time after stress termination. Results are mean ± SE of 10 animals (n = 10) per each group. Significant differences compared with control values are shown by *, $^{\#}P < 0.05$

mice. Recently, stress-induced changes in liver lipid metabolism have been hypothesised to occur in rats (Gao et al. 2013). For example, rats exhibited lower HDL-C and higher LDL-C levels than normal rats in an open-field test that was conducted to induce acute stress (Dunn and Swiergiel 2005).

Based on the hormonal changes in plasma (Figure 3), we suggest that acute stress enhanced the release of CORT and insulin, and attenuated the release of T_3 and T_4. In contrast, no discernible changes in glucagon level were observed as a result of the acute stress (results not shown). These hormonal changes are consistent with previous reports that plasma insulin levels increased in CORT-treated animals (Simon 1984) and that restraint stress increased serum CORT but not glucagon levels in rats (Hidalgo et al. 1986). However, similar to the results shown in Figure 2, the stress-stimulated CORT and insulin values gradually decreased back to the levels of normal mice, whereas the decreased concentrations of T_3 and T_4 persisted during the whole recovery time. In addition, Figure 3 indicates that both stressors induced changes in the parameters to a similar degree, which was also shown in Figure 2. It should also be noted that the changes in the concentrations of plasma parameters over time shown in Figures 1, 2, and 3 might be related in part with non-feeding during the recovery periods.

In addition to insulin and glucagon, thyroid hormones, such as T_3 and T_4 are also important in energy utilisation and metabolism in animals: for instance, T_3 stimulates metabolic rate in cells (Freake and Oppenheimer 1995). However, their relationship with stressors is still controversial. It has been reported that acute stress by single electrical tail-shock decreased peripheral T_3 and T_4 concentrations in adult rats (Helmreich and Tylee 2011). However, Romeo et al. (2007) reported that acute-stress (e.g., a single 30 min session of restraint) does not appreciably influence T_3, T_4, and insulin levels; rather, the developmental stage significantly affects the hormonal levels in rats. We deduce that these discrepancies likely result from different acute stressors and stress intensities, although both of the stressors used in those papers were psychological. The relationship between CORT and energy metabolism is more complicated and the results are conflicting. Buttemer et al. (1991) suggested that corticosterone has no effect on basal

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metabolic rate in birds while elevated plasma corticosterone increased the rate in a terrestrial salamander (Wack et al. 2012).

We also statistically analysed the relationship between stress-induced CORT release and the lipid concentration as well as other hormonal changes using Pearson correlation analysis. Such analysis is commonly used for correlation analysis as a measure of the degree of linear dependence between two variables. This calculated coefficient (r) ranges between +1 and −1, where +1, 0, and −1 represent total (perfect) positive correlation, no correlation, and total negative correlation, respectively (Stigler 1989). These r values varied over the course of the recovery periods and therefore were calculated separately at each indicated time point (points in x-axis of Figures 2 and 3). For example, tape-immobilisation stress induced increases in glucose and CORT levels at 1 h of recovery by approximately 150% and 600%, respectively. The increased values were then used as X and Y variable, or *vice versa*, and r values were calculated at this recovery time. In the same manner, the calculations were repeated at the points for 3, 6, 9, and 12 h. r values were determined over the whole recovery periods; maximum or minimum values among positive or negative numbers, respectively, are presented in Table 2. CORT levels were correlated with the lipid profile including glucose and insulin concentrations. In particular, the r value between total cholesterol and CORT was in the range of 0.433–0.457, which indicates a strong correlation. These results demonstrate that stress-induced CORT may directly regulate plasma lipid metabolism and stimulate the release of insulin into plasma in mice. Although T_3 and T_4 are known to be involved in energy metabolism in animals as mentioned above, the changes in the thyroid hormone levels were not significantly related to plasma lipids, including TG and even glucose. Taken together, these results may also suggest that CORT concentrations are related to changes in circulating lipid, glucose, and insulin levels and that a certain amount of CORT is required to induce lipid changes.

At present, the physiological relevance of the stress-induced increases or decreases in plasma lipids and hormones are still unclear and further studies should be performed to more fully characterise the biological influence of acute stress on plasma parameters. In conclusion, however, the present results collectively elucidate the relationship between stress and the health index of lipids, including hormone levels in plasma.

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