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Changes in Plasma, Liver and Muscle Metabolite Levels in Japanese Quail Exposed to Different Cold Stress Situations

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Summary. 1. Adult Japanese quail, fed *ad libitum*, were subjected to different conditions of cold exposure and winter acclimatization, after which the concentrations of the principal lipid and carbohydrate metabolites were measured in the plasma, liver and muscle.

2. Cold exposure (3.5° C) for two hours produced a marked liver glycogenolysis, but muscle glycogen, plasma glucose and plasma free fatty acid concentrations remained unchanged.

3. Cold exposure (3.5° C) for 21 days produced no change in the concentration of plasma glucose, plasma free fatty acids, muscle and liver glycogen, but the food consumption of quail increased, and quail maintained their body weight.

4. Acute cold exposure (−18° C) for 30 minutes produced hypothermia, marked glycogenolysis in muscle and liver, and increased mobilization of plasma free fatty acids. This was also accompanied by hypoglycaemia in quail which were partially defeathered.

5. Winter acclimatized quail showed a significant hyperglycaemia and a lower plasma free fatty acid concentration, but the levels of muscle and liver glycogen were similar to those of controls.

6. Changes in the concentration of these parameters are discussed in relation to thermogenesis in birds in cold environments.

Introduction

It is well established that exposure to cold increases metabolic rate in birds and mammals, as manifested by increased oxygen consumption and greater thermogenesis. Masoro (1966) stated that fat, carbohydrates and proteins are all involved with thermogenesis, and no single class of fuel is catabolized preferentially. Exposure of chicks (*Gallus domesticus*) to cold causes an increase in oxygen consumption and plasma free fatty acid concentration, and a fall in plasma glucose and hepatic glycogen (Freeman, 1967; Davison, 1973; Palokangas *et al.*, 1973). Davison (1973) also reported elevated plasma uric acid levels in cold exposed chicks, indicating an increased protein catabolism. The large increases in plasma free fatty acid concentration and decrease in respiratory quotient observed in cold exposed chicks are indicative of enhanced lipid oxidation, and this has been attributed to non-shivering thermogenesis (Freeman, 1970a; 1971). Davison (1973) suggested that neonate chicks might maintain deep body temperature by non-shivering thermogenesis, resorting to shivering thermogenesis when heat production from the former process was inadequate, and Freeman (1971) suggested that the liver and kidney might be organs of non-shivering thermogenesis in chicks.

The existence of non-shivering thermogenesis in adult birds is based on indirect evidence (Freeman, 1970b), and has not definitely been shown to occur (Hart,

1962), shivering thermogenesis being generally believed to be responsible for the extra heat production in adult birds (Steen and Enger, 1957; Hart, 1962; Hart and Pohl, 1963; West, 1965). Parker and George (1975) have shown that shivering thermogenesis in the pigeon is largely a glycogenolytic process, heat production being accompanied by intramuscular glycogen depletion. Insofaras different types of substrates have been implicated in the shivering and non-shivering thermogenesis processes, the present study was performed to investigate changes in the concentration of the principal carbohydrate and fat metabolites in the plasma, liver, and muscle of Japanese quail following cold exposure. Three groups of Japanese quail were exposed to cold of different intensity and duration, and a fourth group was acclimatized to outdoor winter conditions.

Materials and Methods

Japanese quail (*Coturnix coturnix japonica*) were reared from eggs produced by a captive colony. After brooding to three weeks of age, the quail were held in battery cages maintained at 20–22° C under a light:dark cycle of 12:12 hrs, until fully grown. At nine weeks of age, the quail to be used in the following experiments were selected according to evenness of body weight and integrity of plumage, and then transferred to individual holding cages. All the quail used were male, were of identical age, and had not been cold exposed prior to the experiments. A commercial diet and water were provided *ad libitum* throughout the period prior to experimentation, and during experiments.

Exposure of Quail to Cold of Different Intensity and Duration

These experiments were conducted in temperature and photoperiod controlled environmental chambers. The quail were caged individually, and held for five weeks at a constant temperature of 21° C and a light:dark cycle of 8:16 hrs. The different cold exposure conditions were: 3.5° C for 21 days, 3.5° C for two hours, and −18° C for 30 min.

The 18 experimental quail to be exposed to 3.5° C for 21 days and the 19 control quail were held under similar conditions in separate chambers at an initial temperature of 21° C. The experimental quail were then subjected to a daily temperature decrease of 2.5° C until 3.5° C had been reached, and were then held at this temperature for a further period of 21 days. The control quail remained at 21° C. The daily food consumption of the experimental and control quail was recorded for seven days prior to the experiment, and throughout the 21 day experimental period. The total body weights of the experimental and control quail were recorded one day before, and at the end of the experimental period. The 19 quail to be exposed at 3.5° C for two hours were taken from the chamber in which they had been held with their controls at 21° C, and transferred to a chamber at 3.5° C for two hrs. Control quail were moved to a different location within the same environmental chamber. Acute cold exposure was accomplished by moving quail from the chamber in which they had been held with their controls at 21° C and placing them for 30 min in a chamber held at −18° C. Control quail were sham-moved. In this experiment, the control and experimental quail were further subdivided according to their being fully feathered or partially defeathered. The partially defeathered quail were birds from which approximtely 1 sq. inch of the dorsal skin directly behind the wings was exposed by cutting the feathers level with the skin. This was done two days before cold exposure. The deep body temperature of the control and experimental quail was measured at 21° C, and after cold exposure, using a modified clinical thermometer inserted into the proctodeum.

Acclimatization of Quail to Outdoor Winter Conditions

Twenty female Japanese quail were hatched from eggs in May, 1973. After brooding, ten were housed in large individual cages in a room held constant at 20–22° C, but illuminated with a natural photoperiod. The other ten quail were transferred to a large outdoor holding pen situated on the University of Guelph campus, where they were kept until January, 1974.

The pen comprised an open area, and an enclosed part which provided direct shelter from snow. However, no supplemental heat was provided. These quail were fed the same commercial diet as the other three groups of quail. On the day quail were sampled, the ambient temperature and the deep body temperature of the winter acclimatized and control quail were recorded.

Sampling Procedure

Experimental and control quail were killed at the end of each experiment by decapitation, and all the quail were killed between 11.00 and 14.00 hrs, the photoperiod being equitemporal about noon. Blood was collected in heparinized tubes, centrifuged immediately, and the plasma removed and cooled. Portions of the pectoralis muscles and liver were quickly deep frozen, and held, in liquid nitrogen, and later analyzed for the glycogen concentration (Carroll *et al.*, 1956). Determinations of the plasma glucose and free fatty acid[1] concentration were made on the freshly prepared plasma. Glucose was assayed enzymatically using the glucose oxidase method (Glucostat Special, Worthington Biochemical Corporation, Freehold, New Jersey), and FFA were measured according to the method of Antonis (1965). Plasma triglycerides were measured (Sigma Technical Bulletin No. 405)in the control and acutely cold exposed quail and in the winter acclimatized and control quail. Plasma uric acid was also measured in the winter acclimatized and control quail by the uricase method (Sigma Technical Bulletin No. 680).

Results

The concentrations of plasma glucose and FFA, and the concentrations of liver and muscle glycogen were similar between control quail and quail cold exposed for 21 days. Although plasma concentrations of glucose and FFA revealed a slight hyperglycaemia and hypolipaemia in the cold exposed quail, the differences were not statistically significant (Table 1). Changes in the body weight of quail following cold exposure were not significant, and the body weight of control quail did not change significantly over the same 21 day period. The amount of food consumed by quail increased significantly ($P < 0.01$) during cold exposure, the mean consumption/bird/day during cold exposure being approximately 32 percent higher than at 21°C.

Quail exposed to 3.5°C cold for two hours showed no significant change in the concentration of plasma glucose, FFA, or muscle glycogen. However, the concentration of liver glycogen was significantly ($P < 0.01$) less in the cold exposed quail than in controls (Table 2).

Acute cold exposure of fully feathered quail produced no significant change in the levels of plasma glucose and plasma triglycerides, but a significant ($P < 0.01$) rise in the plasma FFA. The glycogen content of the liver and muscle from cold exposed quail was largely depleted, the glycogen concentration in both tissues being significantly ($P < 0.01$) lower than their respective control levels. The changes observed in partially defeathered, cold exposed quail were similar in nature to those seen in the fully feathered quail. There was a significant ($P < 0.01$) rise in plasma FFA level. However, the plasma glucose level was significantly ($P < 0.01$) reduced below that in both the control quail, and the fully feathered, cold exposed quail. Furthermore, the elevation of plasma FFA level in the partially defeathered quail was much greater than that produced in the fully feathered quail (Table 3). The deep body temperature of the cold exposed, fully feathered quail was 41.2 ± 0.09°C (S.E.), significantly lower ($P < 0.01$) than the pre-

1 FFA = free fatty acids.

Table 1. Levels of plasma glucose and FFA, and liver and muscle glycogen in control quail and quail exposed to cold (3.5° C) for 21 days. Values are the mean ± 1 S.E

	Plasma glucose (mg %)	Plasma FFA (μ eq/l plasma)	Muscle glycogen (% wet weight)	Liver glycogen (% wet weight)
Control quail at 21° C $n = 18$	336.7 ± 14.7	365.4 ± 49.7	0.146 ± 0.008	0.812 ± 0.188
Quail exposed to cold for 21 days $n = 19$	364.6 ± 11.1	300.3 ± 44.4	0.161 ± 0.011	0.773 ± 0.200

Table 2. Levels of plasma glucose and FFA, and liver and muscle glycogen in control quail and quail exposed to cold (3.5°C) for two hours. Values are the mean ± 1 S.E

	Plasma glucose (mg %)	Plasma FFA (μ eq/l plasma)	Muscle glycogen (% wet weight)	Liver glycogen (% wet weight)
Control quail at 21°C $n = 17$	386.8 ± 11.2	313.7 ± 23.78	0.182 ± 0.013	0.843 ± 0.174
Quail exposed to cold for two hours $n = 19$	383.4 ± 12.3	319.8 ± 17.6	0.163 ± 0.010	0.235 ± 0.050

Table 3. Levels of plasma glucose, FFA, and triglycerides, and liver and muscle glycogen in fully feathered and partially defeathered control and cold exposed (−18° C for 30 mins) quail. Values are the mean ± 1 S.E

	Plasma glucose (mg %)	Plasma FFA (μ eq/ l plasma)	Plasma triglycerides (mg %)	Muscle glycogen (% wet weight)	Liver glycogen (% wet weight)
Fully feathered control quail $n = 10$	361.2 ± 12.9	169.5 ± 25.6	279.7 ± 12.6	0.317 ± 0.030	0.369 ± 0.067
Fully feathered cold exposed quail $n = 6$	370.0 ± 16.0	1039.3 ± 91.1	281.6 ± 62.2	0.018 ± 0.002	0.027 ± 0.008
Partially defeathered control quail $n = 6$	358.3 ± 12.5	369.5 ± 43.7	302.7 ± 42.8	0.340 ± 0.021	0.567 ± 0.150
Partially defeathered cold exposed quail $n = 7$	254.0 ± 23.0	1878.3 ± 184.0	302.9 ± 27.1	0.016 ± 0.002	0.013 ± 0.003

exposure value of 43.0 ± 0.08° C. A greater loss in deep body temperature was produced in the cold exposed, partially defeathered quail, the pre-exposure value of 42.6 ± 0.21° C declining to 40.2 ± 0.38° C ($P < 0.01$).

Winter acclimatized quail showed significant ($P < 0.01$) hyperglycaemia and hypolipaemia, but levels of plasma uric acid and triglycerides were not significantly different from control values (Table 4). The mean total body weight of the winter

Table 4. Levels of plasma glucose, FFA, uric acid and triglycerides, and liver and muscle glycogen in winter acclimatized and control quail. Values are the mean ± 1 S.E

	Plasma glucose (mg %)	Plasma FFA (μ eq/ l plasma)	Plasma tri-glycerides (mg %)	Plasma uric acid (mg %)	Muscle glycogen (% wet weight)	Liver glycogen (% wet weight)
Control, indoor acclimatized quail at 20–22° C $n=10$	339.5 ± 12.6	308.5 ± 38.1	377.3 ± 24.6	11.04 ± 0.79	0.302 ± 0.020	0.532 ± 0.160
Winter acclimatized quail $n=9$	405.8 ± 8.2	154.9 ± 9.5	293.8 ± 16.7	13.18 ± 1.30	0.281 ± 0.039	0.612 ± 0.141

Ambient air temperature at the time of sampling was −8 to −10° C.

acclimatized, and control quail was 123.0 ± 3.4 g, and 117.1 ± 3.9 g, respectively. The mean deep body temperature of the winter acclimatized quail was 42.5 ± 0.1° C, that of the control quail 42.4 ± 0.06° C.

Discussion

The exposure of quail to cold for two hours evoked a metabolic response, as indicated by the reduced glycogen content of the liver. However, no increase in plasma FFA level occurred, suggesting that the response was glycogenolytic, liver glycogen being hydrolysed and released as plasma glucose. The similarity in the metabolite concentrations between quail cold exposed for 21 days and control quail shows that acclimation to the cold condition had occurred, entailing, in part, a higher rate of food consumption, but not an enhanced concentration of plasma FFA. The cold stress at −18° C for 30 min was a thermal stress to which warm adapted quail could not acclimate, the cold exposure having produced a reduction in deep body temperature despite an enhanced metabolic rate. Under these conditions of heat loss, the metabolic response was both glycogenolytic and lipolytic, and the dual response can be attributed to cold *per se* because the partially defeathered quail showed a greater reduction in deep body temperature and a more marked glycogenolysis and lypolysis. The hypoglycaemia in the cold exposed partially defeathered quail indicated that the plasma glucose level could not be maintained, the rate of glucose catabolism exceeding the rate of hepatic glycogenolysis.

Winter acclimatized quail showed hyperglycaemia which could be regarded as a prerequisiste of an enhanced glucose catabolism under winter conditions. However, an explanation of the decrease in plasma FFA concentration of the winter acclimatized quail can not be provided. A similar change in the plasma concentrations of glucose and FFA occurred in the winter acclimatized and control ruffed grouse (*Bonasa umbellus*) (Thomas *et al.*, 1975). The body weights of winter acclimatized quail and quail cold exposed for 21 days were not different in weight from control quail, presumably because the *ad libitum* feeding conditions

enabled quail to ingest more food to compensate for the greater energy expenditure, thereby sparing their body tissues from catabolism. This could explain the similarity in plasma uric acid levels of the winter acclimatized and control quail, and the absence of a low temperature effect upon the plasma triglyceride levels, although this last parameter may well have been influenced by the daily pattern of food intake. However, in quail which were acutely (−18°C) cold exposed, marked changes in plasma FFA concentration were not accompanied by changes in the plasma triglyceride level.

Freeman (1970b) reported that short duration cold exposure (4°C for 1 to 2 hrs) of Japanese quail was accompanied by a rise in plasma FFA level, a fall in plasma glucose, and increased glycogenolysis in the liver and muscle. While changes in the concentration of metabolites are similar in the present study to that of Freeman (1970b), the temperature conditions under which they were induced are different. Thus the changes in metabolite concentration obtained in the study of Freeman (1970b) at 4°C, were seen only at lower temperatures in the present experiment. The plumage of quail exposed at 3.5°C for two hours may have restricted the rate of heat loss to a level at which extra heat production, preceded by hepatic glycogenolysis, may have sufficed. Parker and George (1975) have demonstrated that the integrity of the plumage is vital in preventing a drastic drop in body temperature and loss in muscle glycogen, and a comparison of the fully feathered and partially defeathered cold exposed quail in the present study shows that the integrity of the plumage reduced the fall in deep body temperature, the rise in FFA concentration and the depletion of muscle and liver glycogen.

Freeman (1970b) observed that muscle glycogen levels were maintained during the early stages of cold exposure although liver glycogen levels showed signs of depletion, and it was only during the later stages of cold exposure that muscle glycogen was depleted. This, together with the finding in the present study that after two hours cold exposure only the liver glycogen level was lowered, suggests that under conditions of mild cold exposure hepatic glycogen is preferentially depleted. However, under more severe cold conditions there is a depletion of glycogen in both liver and muscle. Pernod *et al.* (1972), using labelled glucose infusions in cold exposed dogs, concluded that the liver glycogen content may be a limiting factor during acute cold exposure, and reported a shift of carbohydrate metabolism towards muscle glycogen catabolism. Minaire *et al.* (1973) also concluded that despite the major utilization of FFA by cold exposed dogs, the duration of the resistance to cold might be limited by the availability of hepatic glycogen. Similarly Baldwin *et al.* (1973) suggested that liver glycogen could be a more important source of energy than muscle glycogen in rats, and Depocas and Masironi (1960) showed that the increase in energy metabolism was proportional to an enhanced carbohydrate metabolism in rats producing heat by shivering thermogenesis.

The present results and those of Freeman (1970b) are consistent with the view that extra heat production in birds is a consequence of shivering by the pectoral muscles. Shivering thermogenesis would deplete muscle glycogen reserves, but these could be replenished by glycogen synthesis from plasma glucose, whose level, in turn, would be maintained by hepatic glycogenolysis. According to this

scheme, a severe depletion of muscle glycogen would accompany severe depletion of liver glycogen reserves, and, possibly, a decrease in the concentration of plasma glucose. Such was the case in the acutely cold exposed partially defeathered quail.

The pectoral muscles of quail comprise two distinct fibre types (Kaiser and George, 1973), a white type specialized for anaerobic glycolytic metabolism, and a red type specialised for aerobic, predominantly lipid, metabolism (George and Berger, 1966). Vallyathan and George (1969), John and George (1973a) and McKeown *et al.* (1974) have reported increases in the concentration of plasma FFA in pigeons subjected to flight-simulated exercise, and Parker and George (1974) have shown that flight-simulated exercise in pigeons is accompanied by an increased oxidation of fatty acids. Thus under flight conditions, FFA could be considered to be the main energy substrates for the red type of fibre. Shivering can be regarded as a form of exercise in which the amplitude of contraction is less than in non-shivering contraction. However, Parker and George, (1975) have shown that shivering activity in the pectoralis muscles is confined to the glycogen utilizing white fibres, the red fibres, apparently, not being involved. Consequently the elevated plasma levels of FFA observed in the present study and that of Freeman (1970b), may not be related to the shivering of the muscle fibres.

Freeman (1970b) suggested that higher levels of plasma FFA in cold exposed quail might be related to the existence of non-shivering thermogenic mechanisms, but, to date, no conclusive evidence has been produced as to the sites or the biochemistry of non-shivering thermogenesis in birds. Increases in the concentration of plasma FFA need not indicate that there is a greater rate of oxidation of FFA (Masoro, 1968), the amount of FFA oxidized depending also upon the turnover rate and metabolic rate of the fat oxidizing tissues, as well as the plasma FFA concentration (Paul and Holmes, 1973). Himms-Hagen (1965) has reported that the process of cold acclimation in rats is related to a decrease in the level of plasma FFA. If higher plasma FFA concentrations were interpreted as being conducive to a greater rate of FFA uptake by tissues actively engaged in oxidative fat metabolism, then quail which were winter acclimatized and cold exposed for 21 days would have been expected to show higher plasma FFA levels than were observed. Similarly ruffed grouse acclimatized to the same winter conditions showed no increase in plasma FFA levels compared to controls (Thomas *et al.*, 1975). Thus, while there is no doubt that avian tissues can oxidize fats, whether the oxidative processes are capable of high rates of heat production in the absence of shivering thermogenesis, remains unknown.

It is possible that the elevated plasma FFA level observed in quail in the present study and that of Freeman (1970b), and in chicks in the studies of Freeman (1967), Davison (1973) and Palokangas *et al.* (1973), was a consequence of an enhanced rate of calorigenesis, rather than the cause of the calorigenesis. Glucagon is known to have potent glycogenolytic and lipolytic activity in birds (Grande, 1968; Grande and Prigge, 1970; John and George, 1973b; Palokangas *et al.*, 1973). The high rate of glycogenolysis in the acutely cold exposed quail, coupled with a high turnover rate of plasma glucose, may have been induced by a greater rate of glucagon secretion. This would not only stimulate hepatic glycogenolysis, but would also produce an activation of triglyceride lipase in the adipose tissue

and a concomitant rise in plasma FFA level. The exposure of warm acclimated quail to cold (−18°C) could also be considered to be stressful, particularly when hypothermia is induced, and under these conditions ACTH could be released into the circulation. Heald *et al.* (1965) have shown that elevated concentrations of ACTH have a lipolytic effect in the fowl, resulting in plasma hyperlipaemia.

Skulachev and Maslov (1960) observed reduced P:O ratios in the mitochondria isolated from the pectoral muscles of pigeons exposed to cold, and suggested that non-phosphorylating oxidation in the muscle was a thermoregulatory mechanism for heat production. While free fatty acids (particularly oleic acid) have been reported to uncouple rat liver mitochondria (Borst *et al.*, 1962; Wojtczak *et al.*, 1969), the significance of phosphorylation uncoupling mechanism in non-shivering thermogenesis in birds and mammals remains largely undetermined (see reviews by Ismail-Beigi and Edelman, 1970, and Beyer, 1963). Similarly, while an enhanced sodium pump activity has been related to non-shivering thermogenesis in mammals (Stevens, 1973; Stevens and Kido, 1974) its thermogenic role in birds has not been investigated. Although the catabolic significance of high plasma FFA levels can not presently be resolved, another aspect of lipolysis could be of significance, insofar as an increased heat production by a largely glycolytic process is concerned. FFA mobilization is accompanied by the release of glycerol into the plasma. Glycerol can be phosphorylated to L-α-glycerophosphate, which can be converted to 3-phosphoglyceraldehyde. This compound can enter the Embden-Meyerhof pathway, and in its conversion to 1,3-diphosphoglycerate, $NADH_2$ is produced, in addition to the ATP generated during the anaerobic formation of pyruvate. Since reoxidation of 1 mole of $NADH_2$ through the hydrogen transport chain generates 3 moles of ATP, and since 1 mole of ATP is used to phosphorylate glycerol, the diversion of glycerol into the glycolytic pathway would yield ATP. Thus, glycerol catabolism could supplement heat derived from the catabolism of carbohydrate. Should mechanisms exist for the oxidation of FFA, then lipolysis would serve a dual function, providing substrates for both an enhanced anaerobic, as well as aerobic catabolism.

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