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Utilization of biomolecules as fuel energy and their physiological mechanism during migration in birds- A review

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Abstract

Migratory birds undergo physiological and behavioral changes to fuel their high energy demanding migratory flights. They increase their food intake as a part of the preparation for migration which results in increase in their body mass. Fat, carbohydrate and protein acquired from food are stored mainly in the adipose tissue (triglycerides), muscle and liver (glycogen) and body organs (protein) in migratory birds. These stored foods act as fuels to support birds' migratory flights. Dietary carbohydrates and lipids not only provide energy for migration but also help in fattening as carbohydrates can be converted into fat and lipids which can be stored. Lipolysis of adipose-stored fats leads to the production of triglycerides, fatty acids and glycerol, which provide energy for migration. Fats are depleted after long migratory flights and replenished during refueling at the stopover sites.

Being chemically reduced and hydrophobic in nature, fat releases more energy on oxidation as compared to carbohydrate and protein. Due to its high energy-yielding nature, the fat is the preferred fuel to support migration in birds. Migratory birds deposit fat and deplete it during the course of migration. Though, the stored fat acts as the primary source of energy, metabolism of body protein also provides energy for migratory flights. Uric acid in plasma is elevated when protein is catabolized. The metabolism of carbohydrate, stored as glycogen in liver and muscle in migratory birds, produces glucose which also fuels migration. Glucose in migratory birds is maintained at stable levels in plasma and it provides energy only for a flight of short period.

Further, catabolism of carbohydrate and protein results in release of metabolic water which helps the migratory birds to maintain their water balance during long dehydrating flight conditions. Different levels of plasma metabolites in migratory birds act as significant indicators of their physiological and metabolic state. Plasma metabolites also give an idea of feeding, fasting and refueling during migration in birds. The available information is scanty and fragmented about how birds meet their migratory requirements and overcome the physiological challenges encountered during migration. The present review article, therefore, focuses on the biomolecules and their plasma biochemistry during migration in birds.

Key words: Birds, Biomolecules, Energy, Fasting, Fattening, Metabolites, Migration

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Introduction

Many migratory birds undertake extraordinary long flights. They travel thousands of kilometers between their breeding areas in summer and overwintering sites. Birds show uninterrupted bouts of flight for several days during their migration. Such migratory journeys impose tremendous energy demands and birds rely solely on energy stored in their body. They obtain energy from lipids stored as triglycerides in their adipose tissue to support the migratory flight. An increase in body fat stores prior to long-distance flights is an important metabolic feature of migratory birds (Dixit and Sougrakpam, 2013; Dixit *et al.*, 2014). On the other hand, body fattening is generally lacking or to a limited extent, even if it occurs, in non-migratory birds. Since the body fat serves as stored food and fuel during migratory flights in birds, its deposition in non-migratory resident birds, that have an easy access to food in their surroundings throughout, would hamper their flight activities (Dixit and Singh, 2011; 2012, 2020; Dixit *et al.*, 2018). These body stores comprise mainly lipids and proteins (Lindstorm and Piersma, 1993; Piersma *et al.*, 1999). Though migratory birds rely primarily on stored lipids, it is found that plasma uric acid is also elevated in several passerines with stopover migratory flights (Jenni-Eiermann and Jenni, 1991). This shows that catabolism of body protein also occurs during migration (Robin *et al.*, 1987).

Migratory birds maintain their metabolism using body fat, carbohydrate and protein as fuels. Triglycerides are indicators of fat deposition in migrants (Jenni and Schwilch, 2001). Similarly, uric acid is an indicator of protein catabolism (Cherel *et al.*, 1988a; 1988b), whereas β -hydroxy-butyrate indicates fat catabolism and fasting (Cherel *et al.*, 1988a; Jenni-Eiermann and Jenni, 1994). It has been suggested that migratory songbirds may supplement fatty acids with triglycerides in plasma to provide more energy to muscles (Jenni-Eiermann and Jenni, 1992). The studies conducted on migratory birds suggest that the analyses of their plasma metabolites can give a view of their physiological and metabolic states (Guglielmo *et al.*, 2001; Jenni-Eiermann *et al.*, 2002; Gerson and Guglielmo, 2011a; Gerson and Guglielmo, 2013). Gerson and Guglielmo (2013) have suggested that changes in plasma metabolites reveal the metabolic response to migratory flights of various durations and also the effects of humidity conditions during the flight. Profiling of plasma metabolites provides an idea of refueling in migratory birds (Guglielmo *et al.*, 2005).

It also offers a physiological means to assess the instantaneous refueling rate of birds. Also, metabolic states such as feeding and fasting are reflected in the circulating levels of metabolites in the blood (Williams *et al.*, 1999). The frequently asked questions about how birds meet their metabolic requirements and overcome the physiological challenges faced by them during migration remain far from answering satisfactorily, despite some advancement in field. Although literature on bird migration has become voluminous (McWilliams *et al.*, 2004; Barcante *et al.*, 2017; Akesson and Helm, 2020), our knowledge about the biomolecules involved

and their metabolism in the bird migration is still scanty. The present review, therefore, focuses on the biomolecules and their plasma biochemistry during migration in birds.

Nutritional sources and food preferences: Migratory birds schedule their life cycle stages such as reproduction, migration (to the wintering ground and back to the breeding area), moult and overwintering periods in a manner to avoid overlap among above high energy-consuming events (Dixit and Sougrakpam, 2013, Dixit *et al.*, 2014). Each of these life cycle stages has different metabolic requirements. For example, birds become hyperphagic and undergo fattening before migration (Dixit *et al.*, 2017; Dixit *et al.*, 2020). There is a simultaneous increase in gonadal size while preparing for migration as observed in migratory yellow-breasted bunting (*Emberiza aureola*) (Dixit and Sougrakpam, 2013; Dixit *et al.*, 2014). Also, migratory birds build-up body protein during their feathers moult. They alter their preferences for food during migration, for example, they consume more fruit when the fruit is easily available during autumn season (Berthold, 1993; Parrish, 1997; Bairlein, 2002). Carbohydrates and lipids provide high dietary energy as well as they help in fattening because carbohydrates can be easily converted into fat and lipids which can be directly absorbed and stored (Bairlein, 2002).

Further, birds get their required amount of protein due to higher food intake rates while preparing for migration and refueling at the stopover sites, even if the protein content in their food is low (Langlois and McWilliams, 2010; Marshall *et al.*, 2015). Foods containing high-carbohydrates over high-protein is preferred by the yellow-rumped warblers (*Setophaga coronata*), adjusting their food intake seasonally to match changing energy targets for energy sources (Marshall *et al.*, 2015). They store a large amount of fat when they are provided with high-carbohydrate diets compared with high-protein diets (Smith and McWilliams, 2009; Guglielmo *et al.*, 2017). Migratory birds prefer diets containing unsaturated fatty acids over saturated fatty acids (Bairlein, 2002; 2004; Pierce and McWilliams, 2014). When the fat content in diet or food intake is high, fatty acids absorbed from the diet can be directly assimilated into tissues and the fatty acid composition of triacylglycerol and membrane phospholipids respond accordingly (Maillet and Weber, 2006; Price and Guglielmo, 2009). Diet composition may affect the fattening rate and fuel use during resting and feeding periods at migratory stopover sites. Plasma triacylglycerol and very low-density lipoproteins (VLDL) levels are found elevated in migrating songbirds than fasted or fed birds (Jenni-Eiermann and Jenni, 1991; 1992).

The long flights of migratory songbirds between breeding and wintering grounds are energetically demanding and the birds frequently stop to rest and replenish energy. During the course of migration, birds undergo deposition and depletion of both fat and protein reserves (Ramenofsky, 1990; McWilliams *et al.*, 2004; Dixit *et al.*, 2014). A study in the yellow breasted bunting revealed an increase in fat score with the approach of spring migration which was followed by a decrease in their fat score. A gradual

increase in the fat score was observed again in buntings at the time of their autumnal migration (Dixit and Sougrakpam, 2013; Dixit et al., 2014). The rate of replenishing fat and protein stores at stopover sites depends on daily food intake and food quality (Schaub and Jenni, 2001a; Bairlein, 2002). Therefore, indices of the rate of fat and protein storage are used to indicate the quality of stopover sites for migrating birds (Schaub and Jenni, 2001b; Guglielmo et al., 2005). The migratory food reserves are broken down into intermediary molecules before being converted to acetyl-CoA that enters the citric acid cycle leading to synthesis of ATP via electron transport chain and production of energy to be utilized during migratory journey. However, whenever the energy requirement is low as observed during the non-migratory state, the excess food consumed is stored as macromolecules to be utilized at the time of high energy requirement during migration via various metabolic pathways (Fig.1).

Fat deposition and its metabolism in migratory birds: Long-distance migratory flights are fueled by adipose-stored fats. Migratory birds accumulate large amount of fat stores before the start of migration (Dixit and Sougrakpam, 2013; Dixit et al., 2014) and the deposition of fat reaches as high as 50-60% of their total body mass (Guglielmo, 2018). During migration, birds become hyperphagic and eat 20-40% more food than their maintenance levels (Bairlein, 2002; Landys et al., 2005). As a result, they gain body mass at a rate of ~1-7% per day (Alerstam and Lindström, 1990; Lindström, 1991). This is evident in the yellow-breasted bunting that exhibits a significant increase in their body mass and increases in fat scores as they prepare for migration (Dixit and Sougrakpam, 2013; Dixit et al., 2014). Fats, universal storage forms of energy in living organisms, are derivatives of fatty acids.

Fatty acids found in the organisms usually belong to three classes of esters: triglycerides, phospholipids and cholesteryl esters. Fat is mainly stored in the form of triglycerides in migratory birds. A triglyceride molecule is an ester of three fatty acids connected to a glycerol unit. Triglycerides are synthesized in the liver and then transported to peripheral tissues for their deposition in fat bodies. However, they can also enter the blood through absorption from food (Robinson, 1970). Fatty acids are synthesized *de novo* in the liver mainly from glucose when birds get more dietary energy than their energy demands (Klasing, 1998). A major amount of fat is stored in sub-cutaneous adipose tissue (Berthold, 1993; Mailletand Weber, 2006). Fat is also stored in flight muscles (pectoralis), liver, mesenteries and connective tissue in the abdominal cavity, sometimes enveloping the intestine (Mailletand Weber, 2006; Guglielmo, 2018). These stored fatty acids are transported and oxidized at very high rates in the migratory birds to sustain flights for many hours or days.

Birds rely more on hepatic and muscular lipids during their short fasting periods (Jenni-Eiermann and Jenni, 1996). Fatty acids are stored as triglycerides which are almost anhydrous, containing only 5% water. On the other hand, carbohydrates (stored in liver and muscle as glycogen) and protein stores contain a higher amount of water (70-80%) (Jenni-

Eiermann et al., 2001). Fat contains eight to ten times more energy than other fuel types such as carbohydrates and protein. Being chemically more reduced than carbohydrate and protein (amino acids), fatty acids yield more energy when oxidized. As fat provides eight times more energy per unit wet mass (~37 kJg⁻¹) than carbohydrates or protein (~4-5 kJg⁻¹), it acts as the most preferred fuel for migration in birds. This production of more energy upon oxidation is possible because fat is highly reduced and fatty acids are hydrophobic in nature (Jenni and Jenni-Eiermann, 1998). Also, lipid imposes a low degree of wing loading in migratory birds as compared to other fuels such as carbohydrate or protein (Schmidt-Nielsen, 1990).

Birds derive energy from lipids stored in the form of triglycerides in adipose tissue to support the migratory flight (Ramenofsky, 1990). This required energy for migratory flight is derived from the mobilization of lipids. In passerine migrants, the elevation of two breakdown products of triglycerides *i.e.*, fatty acids and glycerol in plasma, are found to be associated with flight (Jenni-Eiermann and Jenni, 1991). It has been found that plasma triglycerides levels are elevated during the initiation of migration (Jenni and Jenni-Eiermann, 1996; Guglielmo et al., 2002a; 2002b). A study conducted by Jenni-Eiermann et al. (2002) on migratory red knots, *Calidris canutus* flying in a wind tunnel under controlled conditions showed that the fat breakdown products are elevated significantly with flight. Plasma triglycerides are supplemented by plasma fatty acids to provide energy for working muscles of migratory songbirds with high mass-specific metabolic rates (Jenni-Eiermann and Jenni, 1992). Plasma triglyceride is an indicator of fat deposition in migratory birds. The concentration of plasma triglyceride increases during feeding and fat deposition because of absorption of lipids in the gut or *de novo* synthesis in the liver (Guglielmo et al., 2005). It has been suggested that small passerine birds use very low-density lipoproteins high in triglycerides to transport fatty acids in high concentration through the blood during migratory flight (Jenni and Jenni-Eiermann, 1992; Price, 2010). An increase in triglycerides levels during migration varies with the size of birds. Triglycerides levels are elevated early in birds which are smaller in size as compared to large ones.

Small migratory birds caught in the wild show increased triglycerides during migratory flight, but larger birds and birds flown in wind tunnels show reduced or stable plasma triglycerides (Jenni-Eiermann and Jenni, 1992; Jenni-Eiermann et al., 2002). Triglycerides and lipoprotein transport pathways are not up-regulated during migratory flights in larger passerine birds e.g. American robin (Gerson and Guglielmo, 2013). However, triglycerides may play a role in fat transport during long-distance migration as large quantity of lipids are present in circulating triglycerides (Landys et al., 2005). Also, Landys et al. (2005) conducted a study on bar-tailed godwits, *Limosa lapponica* on arrival at their stopover area to determine the utilization of fat substrates and protein breakdown to support flight. Their study revealed the importance of lipid stores to support long-distance migration. The plasma levels of triglyceride were found to decrease in arriving godwits inactive for a long time.

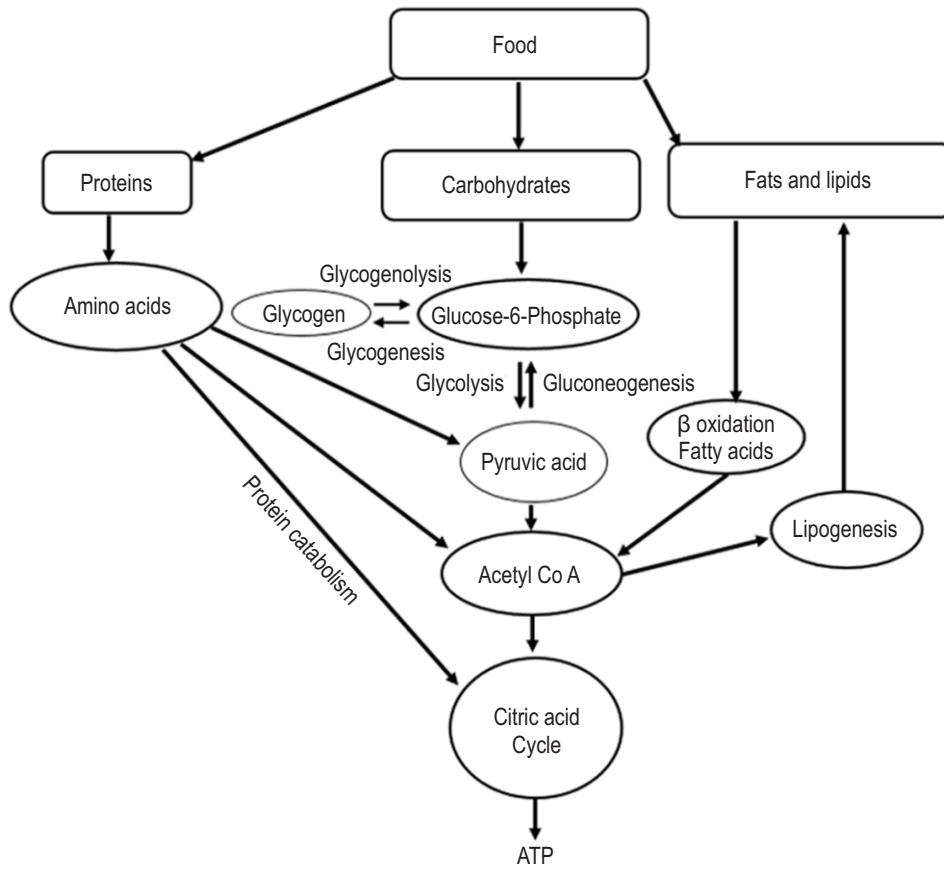


Fig. 1: Interlinking pathways of protein, carbohydrate and lipids metabolism.

This suggests that elevations of plasma levels of triglyceride are associated with migratory flight. Plasma triglycerides in migrants increase during pre-migratory fattening and during refueling at stopover sites (Jenni-Eiermann and Jenni, 1996; Guglielmo *et al.*, 2002a). Changes in triglycerides levels in plasma are positively correlated with body mass gain in many migrants (Jenni-Eiermann and Jenni, 1994; Williams *et al.*, 1999; Jenni and Schilch, 2001). Landys *et al.* (2005) had found that levels of triglycerides were higher in godwits in advanced-refueling stage as compared to initial-refueling stage.

This suggests that migratory birds gain mass during the later refueling stage of stopover (Dixit and Sougrakpam, 2013). Plasma triglyceride levels are high during the stages of high body mass in migratory red knots (Jenni-Eiermann *et al.*, 2002). More triglycerides are available after food intake in passerines during periods of fat deposition (Jenni-Eiermann and Jenni, 1996). β -hydroxy butyrate is an indicator of fasting and lipid utilization in migratory birds (Guglielmo *et al.*, 2005). When birds undergo fasting and consequent loss of body weight, glycerol level increases due to high rate of lipolysis and β -hydroxy butyrate level increases due to high rate of ketone formation. Studies on captive birds show that triglycerides, glycerol and β -hydroxy butyrate can

be used to predict change in the body weight (Williams *et al.*, 1999; Jenni and Schilch, 2001). β -hydroxybutyrate, being an indicator of fat catabolism, has been found to be negatively correlated to gain in body mass (Jenni-Eiermann and Jenni, 1994; Jenni and Schilch, 2001). Higher concentrations of β -hydroxybutyrate, a non-esterified fatty acid, are indicators of high rates of fat mobilization during migratory flight or exercise (Jenni-Eiermann *et al.*, 2002). A study by Jenni-Eiermann *et al.* (2002) revealed that β -hydroxy butyrate levels are negatively correlated to body mass change in long-distance migratory birds. Field studies show that metabolic profiles are different in different stopover sites during migration suggesting the differences in refueling rates (Schaub and Jenni, 2001b; Guglielmo *et al.*, 2002a). Elevated levels of plasma phospholipid may reflect fat deposition in free-living birds (Guglielmo *et al.*, 2002b).

Plasma phospholipid in combination with triglycerides may provide information about lipid composition of the diet (Guglielmo *et al.*, 2002b). A study on western sandpipers (Guglielmo *et al.*, 2002b) showed that concentrations of plasma phospholipid can become very high (higher amounts of fatty acids than plasma triglycerides) during stopover refueling. This indicates that dietary phospholipid may be absorbed and

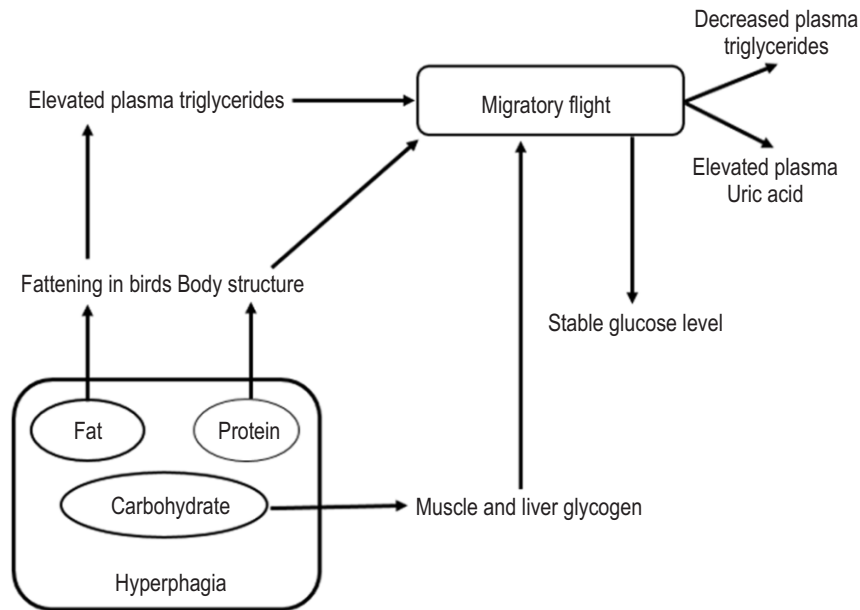


Fig. 2: Diagrammatic presentation of biomolecules and their plasma metabolites during migration.

transported in this form. Guglielmo *et al.* (2005) conducted a field study on American robin (*Turdus migratorius*), gray catbird (*Dumetella carolinensis*), hermit thrush (*Catharus guttatus*), Swainson's thrush (*Catharus ustulatus*), magnolia warbler (*Dendroica magnolia*) and white-throated sparrow (*Zonotrichia albicollis*). These six passerine bird species were selected on the basis of their refueling sites categorized as high-quality and low-quality sites. This study revealed that plasma phospholipid had positive correlation with triglycerides in all species indicating that some dietary or endogenously synthesized lipids are transported in this form. Jenni-Eiermann and Jenni (1992) proposed that triglycerides increase the concentration of circulating fatty acids in plasma, once albumin gets saturated with non-esterified fatty acids (NEFA).

Glycerol is considered as an indicator of fatty acid mobilization as it is released by adipocytes during lipolysis (Jenni-Eiermann and Jenni, 1991; Williams *et al.*, 1999; Guglielmo *et al.*, 2002a). In captive western sandpipers, it was found that plasma glycerol increased with decrease in body mass and vice-versa that indicates poor feeding conditions (Williams *et al.*, 1999). Guglielmo *et al.*, (2005) inferred a U-shaped pattern of glycerol and triglycerides relationship. From this pattern, it can be concluded that glycerol is negatively related to triglycerides when the concentration of triglycerides is low to moderate. But the concentration of glycerol increases with an increase in the concentration of triglycerides when the concentration of triglycerides was 4mmol l^{-1} or above. This occurred probably due to the increasing glycerol production during lipolysis at low triglycerides concentration and during rapid uptake of fatty acid by adipose tissue and muscle when plasma triglyceride was very

high. When triglyceride is hydrolyzed by adipose and muscle lipoprotein lipase at the endothelial surface of the cell, glycerol is released in the plasma. That is why, extremely high rates of fat deposition may result in elevated plasma glycerol. Levels of glycerol are indicators of high rates of fat mobilization during migratory flight or exercise (Jenni-Eiermann *et al.*, 2002). A study by Landys *et al.* (2005) on bar-tailed godwits subjected to 5h of inactivity on their arrival, shows that levels of plasma free fatty acids and glycerol decrease. These findings suggest lipolysis of stored lipids in migrants during their migratory flight. On the other hand, plasma levels of free fatty acids and glycerol were found elevated in birds just arrived at the stopover site when compared with refueling birds. Migratory birds may be considered as machines for burning fats. Fatty acids are stored in adipose tissue (triacylglycerol), which work as fuel for birds during their migratory flight. Flight muscles of migratory birds have mitochondrial enzymes such as hydroxyacyl-CoA-dehydrogenase (HOAD) and carnitine palmitoyl transferase (CPT) which are involved in fatty acid oxidation (Lundgren and Kiessling, 1985).

These enzymes increase during migratory seasons (Guglielmo *et al.*, 2002a; Banerjee and Chaturvedi, 2016). Further, these enzymes may also increase at the stopover sites as birds refuel and prepare for departure (Driedzic *et al.*, 1993; Mailetand Weber, 2007). Many migratory birds show two peaks of migratory body fattening in a year, first during spring and later during autumnal migration (King, 1970; Dixit and Sougrakpam, 2013). In migrating songbirds, fatty acids are mobilized from adipocytes and may be cleared from the plasma by the liver. These fatty acids are esterified to triacylglycerol (and possibly phospholipids), and packaged in the form of very low density lipid

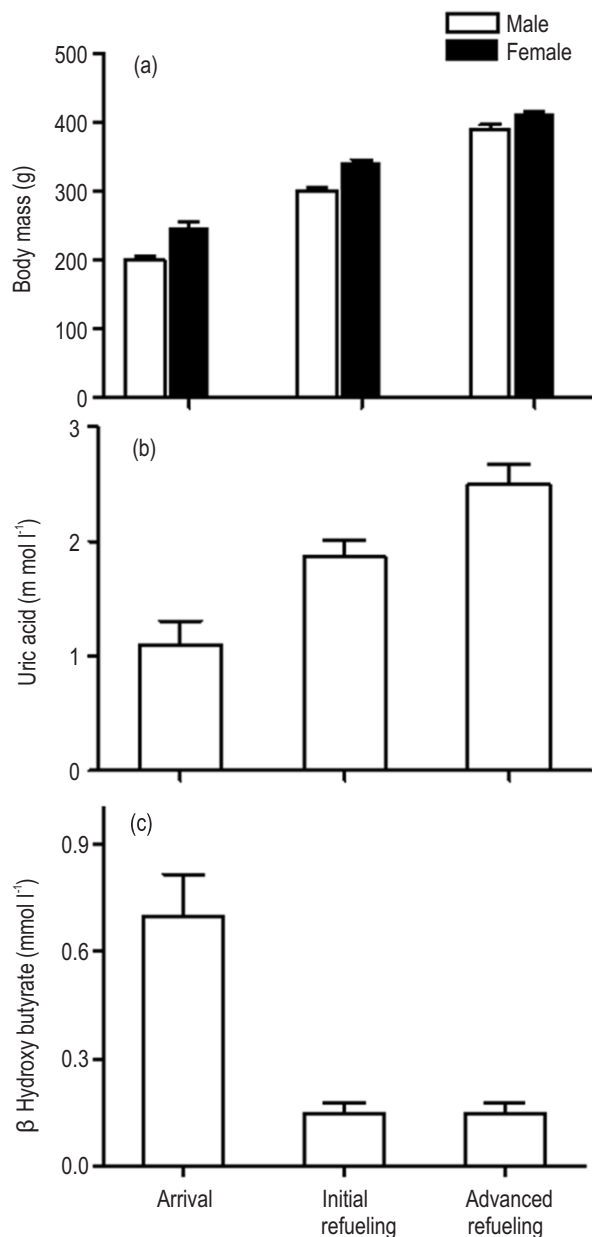


Fig. 3: Body mass (a) and plasma levels of uric acid (b) and β -hydroxybutyrate (c) of godwits during different stages of migration (redrawn as per Landys *et al.*, 2005).

and are released to the blood for circulation in the body (Jenni-Eiermann and Jenni, 1992). Migratory birds undergo hyperphagia before the onset of migration that leads to deposition of large amount of fat and relatively smaller amounts of protein and carbohydrate in various tissues resulting in an increase in body mass. The fats, as main reserved food, are stored mainly in the adipose tissues. During migration, the increased plasma triglycerides are catabolized to support migratory flight.

Triglyceride levels in plasma fall after a long flight. Carbohydrates stored as glycogen in muscles and liver and protein stored in flight muscles and body organs also degrade to produce energy, particularly during early stages of flight (Fig. 2).

Metabolism of protein: Protein is stored in large quantity as body structure in living organisms. Proteins from muscles and digestive organs are used during migratory flights (McWilliams and Karasov, 2001). It is suggested that catabolism of body protein also occurs in migratory birds (Mori and George, 1978, Robin *et al.*, 1987). Digestive organs, which have greater protein turnover rates than muscle, catabolize proteins during long flights in migratory birds (Battley *et al.*, 2000; Bauchinger *et al.*, 2005; Bauchinger and McWilliams, 2009). Catabolism of protein leads to the reduction of flight muscle size and loss of body mass over a flight (Jenni and Jenni-Eiermann, 1998; Pennycuik, 1998). This reduction in body mass makes flight easier for migratory birds. Breakdown of body tissue or food originated proteins produces uric acid (Robin *et al.*, 1987; Lumeij and Remple, 1991; Lindgard *et al.*, 1992). Elevations of plasma uric acid are reported in several short-flying passerines during migration (Jenni-Eiermann and Jenni, 1991). Protein contains higher amount of water and it acts as a source of supplemental water under dehydrating migratory flight conditions (Jenni and Jenni-Eiermann, 1998; Gerson and Guglielmo, 2011a).

Breakdown of protein liberates metabolic water for the maintenance of water balance in migratory birds (Bintz and Strand, 1983). Thus, though migrants rely primarily on lipid stores, at times they use energy from catabolism of proteins. A study on migratory shorebirds flying in a wind tunnel revealed that plasma uric acid increases during flight. Changes in the lean, dry mass of long-flying migrants also suggest that migratory species utilize proteins during their migratory flight (Battley *et al.*, 2000; Bauchinger and Biebach, 2001). The uric acid levels decrease in arriving godwits subjected to forced in activity at the stopover sites (Landys *et al.*, 2005; Fig. 3). This findings suggest that higher amount of protein is catabolized in flying migrants as compared to inactive fasting birds. It has been proposed that protein breakdown may not only maintain intermediates of citric acid cycle which are necessary for fatty acid oxidation, but may also give energy to muscles through gluconeogenesis (Viega *et al.*, 1978; Dohm, 1986). Migratory godwits flying for two days do not show a lack of dehydration (Landys *et al.*, 2000).

It indicates that catabolism of protein provides metabolic water to migrating birds. Godwits refueling at stopover sites show higher levels of plasma uric acid as compared to godwits that arrived at the stopover. It indicates that dietary protein is metabolized to derive energy or protein is transformed into lipid (Cherel *et al.*, 1988a; Lumeij and Remple, 1991). Jenni-Eiermann *et al.* (2002) carried out a study on long-distance migrant shorebird red knot, *Calidris canutus* under thermal and photoperiodic conditions of their temperate wintering area. They measured plasma levels of triglycerides, β -hydroxybutyrate and uric acid. Their study showed that the plasma uric acid levels were

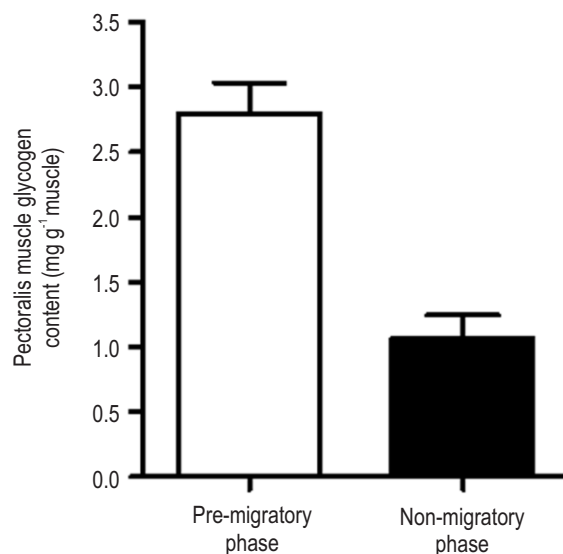


Fig. 4: Glycogen levels of pectoralis muscle during pre-migratory and non-migratory phases in red-headed bunting (redrawn as per Banerjee and Chaturvedi, 2016).

positively and β -hydroxy-butyrate levels were negatively correlated with body mass changes (Fig. 3). High uric acid levels of red knots gaining body mass can be interpreted as increased intake and breakdown of proteinaceous nutrients. Uric acid can be elevated during re-absorption of nutritional proteins (Jenni-Eiermann and Jenni, 1991; 1994) which reflects the breakdown of these proteins. Uric acid levels can also be elevated at the time of long-term fasting reflecting body protein breakdown (Cherel *et al.*, 1988a; 1988b). Further, plasma levels of uric acid increase when protein catabolism is high (Gerson and Guglielmo, 2011a; Gerson and Guglielmo, 2011b).

Metabolism of carbohydrate: Migratory birds use glucose from muscle and liver stored in the form of glycogen. It is important in migratory birds for burst flight to escape predators. Glucose is the primary carbohydrate absorbed by the intestine of birds (Klasing, 1998). It is important during the early stages of migratory flight when fatty acid mobilization is induced (Gerson and Guglielmo, 2013). Migratory birds store a too small amount of glycogen to support hours or days long migratory flights (Driedzi *et al.*, 1993; Banerjee and Chaturvedi, 2016). Studies on semipalmated sandpipers, *Calidris pusilla* (Driedzi *et al.*, 1993) and western sandpipers *Calidris mauri* (Maggini *et al.*, 2017) show that pectoralis muscle glycogen can fuel flight for less than five minutes.

The concentration of pectoralis glycogen in gray catbird has been found threefold higher than in sandpipers with high liver glycogen levels. But these glycogen stores provide energy only for a flight of short period (Fig. 4). Amino acids from organs and muscle proteins are used as gluconeogenic precursors to maintain the glucose levels in blood and metabolites, which are important for citric acid cycle (Jenni and Jenni-Eiermann, 1998).

Migratory birds show stable glucose level during flight and glucose level in blood is regulated within narrow limits (Jenni-Eiermann and Jenni, 1991; Jenni-Eiermann *et al.*, 2002; Landys *et al.*, 2005). Gluconeogenesis helps migratory birds to maintain glucose levels in blood plasma. Glucose is also substituted by butyrate in migrants (Landys *et al.*, 2005).

Migratory birds become hyperphagic to store fuel as a source of energy to support migratory flights as a part of the preparation for migration. The major amount/chunk of above stored fuel comprises body fat. Simultaneously, protein is also stored in various body organs leading to increase in their size and carbohydrate is stored as glycogen in muscle and liver. This results in an increase in the body mass of birds. Fats provide more energy per unit wet mass as compared to carbohydrate or protein. Blood plasma levels of triglycerides are elevated during pre-migratory fattening. The body fat stores in birds get depleted after long migratory flights and are replenished quickly during stopover. Lipolysis of stored lipids occurs during migratory flight to release energy. Plasma levels of triglycerides goes down after long flights in migrants, but levels of fatty acids and glycerol rise up. In addition, catabolism of carbohydrates and protein also takes place particularly during the early phase of flight.

The switch over from the catabolism of carbohydrate and protein to fat is faster in migratory birds due to enhancement in their ability to mobilize and catabolize fat. Carbohydrate and protein catabolism not only provides energy during the early stages of flight, but also yield approximately five-times more metabolic and bound water than catabolism of fat which is utilized by the birds. Utilization of this water and excretory water losses help them to reduce average body mass. This reduction of the

body mass of migratory birds helps them to extend their flight range and reduce flight costs as the wing load is also reduced. Catabolism of protein and resultant lean mass further helps in maintaining metabolic and osmotic homeostasis in migratory birds. Profiling of circulating metabolites in the blood give an idea about physiological and metabolic states of migratory birds. It also provides information about refueling and feeding and fasting. Thus, the concentrations of different plasma metabolites in migratory birds may be used as indicators to determine their physiological and metabolic states, refueling at stopover sites and feeding and fasting as a part of future research. Further, the investigations directed to roles of different hormones namely, ghrelin and leptin in inducing migratory states would be interesting.

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Add-on Information

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