Increased lipogenesis in isolated hepatocytes from cold-acclimated ducklings

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Thermogenic endurance and development of metabolic cold adaptation in birds may critically depend on their ability to synthesize and use fatty acids (FA) as fuel substrates. Hepatic lipogenesis and the capacity to oxidize FA in thermogenic tissues were measured in cold-acclimated (CA) ducklings (Cairina moschata) showing original mechanisms of metabolic cold adaptation in the absence of brown adipose tissue, the specialized thermogenic tissue of rodents. The rate of FA synthesis from [U-14C]glucose and from [1-14C]acetate, measured in incubated hepatocytes isolated from 5-wk-old thermoneutral (TN, 25°C) or CA (4°C) fed ducklings, was higher than in other species. Hepatic de novo lipogenesis was further increased by cold acclimation with both glucose (+194%) and acetate (+111%) as precursor. Insulin slightly increased (+11–14%) hepatic lipogenesis from both precursors in CA ducklings, whereas glucagon was clearly inhibitory (−29 to −51%). Enhanced de novo lipogenesis was associated with higher (+171%) hepatocyte activity of glucose oxidation and larger capacity (+50 to +100%) of key lipogenic enzymes. The potential for FA oxidation was higher in liver (+61%) and skeletal muscle (+29 to +81%) homogenates from CA than from TN ducklings, suggesting that the higher hepatic lipogenesis may fuel oxidation in thermogenic tissues. Present data underline the high capacity to synthesize lipids from glucose in species like muscovy ducks susceptible to hepatic steatosis. Lipogenic capacity can be further increased in the cold and may represent an important step in the metabolic adaptation to cold of growing ducklings.

malic enzyme; glucose-6-phosphate-dehydrogenase; fatty acid synthase; acetyl-CoA carboxylase; glucagon; insulin; thermogenesis; fatty acid oxidation

Survival of endotherms in the cold critically depends on their ability to sustain increased levels of energy expenditure for long periods and thus requires sustained substrate mobilization from the main energy stores, i.e., adipose tissue. Constitution and replenishment of fat stores through de novo lipogenesis will therefore critically affect thermogenic endurance and survival in the cold (33, 41). In rodents, for instance, stimulation of de novo lipogenesis in liver and thermogenic brown adipose tissue (BAT) (21, 44) is an essential step of cold acclimation. Regulation of lipogenesis and lipid fueling has received considerably less attention in birds, another class of endotherms showing high cold endurance (5, 41), yet it might be anticipated that adaptations of lipid metabolism may be more critical in birds than in mammals. Indeed, birds have relatively higher metabolic rates, are subject to intensive aerobic exercise through flying, and often undergo seasonal migrations mostly relying on lipid substrates (review in Ref. 31). Birds therefore provide useful comparative models for investigating the adaptations of lipid metabolism under energetic constraints especially in those species showing high lipogenic capacity and likely to develop steatosis such as muscovy ducks (Cairina moschata) or Landes geese (Anser anser) (30). Furthermore, some bird species, including the muscovy duck, can increase their thermogenic capacity after cold acclimation through the development of original mechanisms of muscle-nonsivering thermogenesis in the absence of BAT, and fatty acids (FA) are essential in these mechanisms (3, 17, 18, 38).

Cold-induced adaptation of lipid metabolism has been investigated in ducklings. It was shown that cold enhanced supply of FA to thermogenic muscles through 1) an increased food intake (+30%; Ref. 7), 2) an increased lipolytic activity in adipose tissue (3, 7), and 3) an increased uptake of circulating triglycerides by the activity of lipoprotein lipase (7). Parallel improvement of de novo lipogenic activity to directly fuel thermogenic tissues or contribute to replenish stores has not been documented so far in cold-acclimated (CA) birds.

In birds, as in humans, de novo lipogenesis mainly, if not exclusively, occurs in liver (22), whereas in rodents, both liver and adipose tissue are lipogenic tissues (re-

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view in Ref. 27). In vitro incorporation of labeled glucose or acetate into lipids can be investigated with isolated hepatocytes as was done for instance in Japanese quails and chicken (27, 35). Compared with mammals, glucose is used extensively and is a major substrate for hepatic lipogenesis (35). Lipogenesis is slightly upregulated by insulin and markedly down-regulated by glucagon (23). A putative improvement of lipogenesis in the cold may thus depend on a change in acute hormonal control of hepatocyte activity and on an improved enzymatic capacity of the hepatocyte. Lipogenesis depends on the activity of key cytosolic enzymes involving 1) acetyl-CoA carboxylase (ACCx), which transforms acetyl-CoA to malonyl-CoA, 2) fatty acid synthase (FAS), which catalyzes the production of medium chain FA from malonyl-CoA, 3) malic enzyme (ME), and 4) glucose-6-phosphate dehydrogenase (G6PDH), which both supply the required reduced equivalent (NADPH) (24, 26, 45).

The aim of this work was to investigate whether hepatic lipogenesis and the capacity to oxidize FA in thermogenic tissues were improved in CA ducklings showing metabolic adaptation to cold. In vitro de novo lipogenic activity was measured by the incorporation of [U-14]glucose and [1-14]acetate into FA in hepatocytes isolated from 5-wk-old thermoneutral (TN) control or CA ducklings. Cells were obtained from fed birds to favor lipogenic conditions and were incubated in the presence or absence of insulin and glucagon to assess possible changes in hormone responsiveness. In parallel, activities of ACCx, G6PDH, and ME, involved in the short-term modulation of lipogenesis, as well as the activity of FAS, involved in the rather long-term regulation of lipogenesis, were determined in liver homogenates. Finally, the potential for FA oxidation was measured in liver and skeletal muscle homogenates from TN and CA ducklings.

MATERIALS AND METHODS

Animals. Male Muscovy Ducklings (Cairina moschata, pedigree R51) were obtained from the age of 1 day from a commercial stockbreeder (Ets Grimaud). The cold acclimation protocol described previously (3) was used. From the age of 1 wk, ducklings were caged for 4 wk at either 4°C ambient temperature (CA ducklings) or 25°C (TN controls). Birds were fed a commercial mash (Aliment Genthon Démarrage, Genthon 5 A) containing 18.4% proteins and 3.1% lipids. “Guiding Principles For Research Involving Animals” were followed (1).

Birds were kept in a constant short photoperiod (8 h of light and 16 h of darkness) to enhance the development of metabolic cold acclimation (39) and were allowed free access to food and water. All animals were kept at thermoneutrality (25°C) with no access to food for at least 1.5 h before the start of the surgery to switch off thermogenic processes and to have ducklings in a similar state. At experiment, ducklings weighed ~1.2–1.7 kg.

Isolated hepatocytes. Hepatocytes were isolated by liver perfusion with collagenase by an adaptation of the method described for rats by Berry and Friend (8). Isolation and perfusion of duckling liver were performed as described previously (6). The liver was first perfused 15 min at constant flow rate (38 ml/min) with a Krebs-Heinselteit calcium-free bicarbonate buffer (pH 7.4) containing 20 mM glucose and 3.3 mM HEPES. At the start of the perfusion, the liver was excised out of duckling carcass. The perfusion then continued for 15 min with recirculation of the buffer, in which 0.025% collagenase (type IV, Sigma Aldrich) and 1.3 mM CaCl2 were added. All buffers were kept at 40°C and continuously gassed with carbogen (95% O2-5% CO2).

At the end of the perfusion, connective tissue and large blood vessels were removed, and the liver was minced. The slurry was oxygenated for 2 min in a shaken Erlenmeyer flask. Cells were then filtered gently through a 500-µm nylon mesh and spun at 50 g for 2–3 min. The supernatant was discarded and cells were washed three times with a Krebs buffer containing 2.4 mM CaCl2 (pH 7.4, 4°C). Isolated hepatocytes were then filtered, gassed with carbogen, and kept on ice (4°C) for 30 min before use to allow a good recovery of cytoplasmic nucleotides (15). At the end of the isolation, cell viability was regularly estimated by trypan blue exclusion and was always greater than 90%. Incubations of hepatocytes in closed vials. Lipogenesis was classically measured by incorporation of radiolabeled substrates (acetate and glucose) as described by others (23, 35). Acetate allows a direct labeling of cytosolic acetyl-coenzyme A, the immediate precursor for de novo FA biosynthesis, while glucose exchanges with different cellular pools before being used for FA synthesis (review in Ref. 26). Cells (10 mg dry weight cells/ml) were incubated in glass flasks (25 ml) with oxygenated (95% O2-5% CO2) Krebs-Heinselteit bicarbonate buffer (pH 7.4) containing 2.4 mM CaCl2, fatty acid-free BSA 0.5%, 10 mM glucose, 20 mM HEPES, 2.5 mM acetate, 10 mM alanine, and [1-14]acetate (20 µCi/mmol, ICN Pharmaceuticals) or [U-14]glucose (7.4 µCi/mmol, NEN Life Science Products). As in quail (35), addition of alanine improved lipogenic activity in duckling hepatocytes. Incubations were run in duplicates. Flasks, which were saturated with 95% O2-5% CO2 and closed with a rubber stopper, also contained a small CO2 trap. This trap allowed measurement of 14CO2 production and thus glucose oxidation. Cells were incubated at 40°C in a shaking water bath for 30, 60, or 90 min. Lipogenesis and glucose oxidation were measured in basal conditions and in the presence of 1 nM glucagon (Glucagen, NOVO Nordisk) or 100 nM insulin (Actrapid, NOVO Nordisk).

Incubations were stopped by addition of 5 N HClO4. Then 200 µl ethanolamine-ethylene glycol (1:2 vol/vol) were introduced in the filter trap through the rubber stopper, and flasks were replaced in the shaking water bath for 1.5 h to ensure complete entrapment of 14CO2. Lipids were then extracted (20) and purified by chloroform/methanol (2/1 vol/vol), washed three times, and then dried under nitrogen. After methylation, the FA fraction (essentially issued from triglycerides and phospholipids) was extracted by hexane and accounted for 97% of the radioactivity incorporated into the lipid fraction.14CO2 incorporated into FA and trapped CO2 was counted in a β-lipid liquid scintillation spectrometer, in 5 ml of Picofluor and 8 ml of Hionicfluor, respectively (Packard Instruments), using quenching correction by external standard.

Enzyme analysis. Activity of key lipogenic enzymes was determined in liver homogenates of another batch of ducklings. Tissue was homogenized in 0.25 M sucrose buffer and spun at 30,000 g for 40 min at 4°C. The supernatant was analyzed for G6PDH (EC 1.1.1.49) and ME (EC 1.1.1.40) activities using spectrophotometric measurement of NADPH appearance at 340 nm during 4 min (19, 28). G6PDH activity was determined in the presence of diglycine buffer (1.25 M, pH 7.4), 0.1 M MgCl2, 13.9 mM NADP, and 0.2 M glucose.
6-phosphate. ME activity was determined in the presence of triethanolamine buffer (0.4 M, pH 7.4, 0.12 M MnCl2, 3.4 mM NADP, and 0.05 M malic acid. FAS (EC 2.3.1.85) activity was also measured using spectrophotometric method by determining the disappearance of NADPH in the presence of acetyl-CoA and malonyl-CoA at 340 nm during 10 min (13). ACCx (EC 6.4.1.2) was assayed from supernatants by the acetyl-CoA and malonyl-CoA at 340 nm during 10 min (13). Enzymatic kinetic was monitored for 2 and 4 min and stopped by acidification. After evaporation to dryness at 37°C (Rotavapor Buchii), labeled residue was dissolved in 400 μl H2O and counted in 5 ml Hionicfluor. Protein content was assayed by the bicinchoninic acid method and BSA as standard (40).

Oleate oxidation assay. To compare the potential for FA oxidation in the most important thermogenic tissues, liver and skeletal muscle (red and white gastrocnemius muscle) samples were obtained just after killing another batch of ducklings. Tissues were used to measure oleate oxidation rates in vitro (32). Briefly, tissues were rapidly homogenized with a Teflon glass homogenizer in ice-cold buffer (0.25 M sucrose, 2 mM Na2-EDTA, 10 mM Tris-HCl, pH 7.4). Then oleate oxidation was measured in 25 mM sucrose, 75 mM Tris-HCl (pH 7.4), 10 mM K2HPO4, 5 mM MgCl2, 1 mM Na2-EDTA, 1 mM NAD+, 5 mM ATP, 25 μM cytochrome-c, 0.1 mM coenzyme A, 0.5 mM L-malate, and 0.5 mM L-carnitine. Peroxisomal FA oxidation was determined in the presence of mitochondrial oxidation inhibitors (70 μM antimycin A and 10 μM rotenone). All assays were made in duplicates. Preliminary trials showed that inhibitor concentrations adequately blocked mitochondrial oxidation in duckling tissues. Flasks were preincubated for 5 min at 40°C before addition of 100 μl of 600 μM [1-14C]oleate bound to albumin (5:1 molar ratio). Incubation with shaking was carried out for 30 min at 40°C and stopped by 0.2 ml of 3 M perchloric acid. Released 14C was the acid incubation mixture was spun at 10,000 g for 5 min, and the radioactivity of 150 μl supernatant containing 14C-labeled peroxisomal acid-soluble products was measured. Oleate oxidation rates were measured from the sum of 14CO2 and 14C-labeled peroxisomal acid-soluble products (32).

Statistical analysis. Statistical significance was determined using ANOVA for repeated or factorial measurements, and the different means were analyzed by post hoc tests (Fisher least significant difference). Significant differences were recognized at P < 0.05. Values are expressed as means ± SE. The global effect of hormones on hepatocyte incorporation of acetate or glucose into lipids and CO2 release during incubations was estimated by calculating the area under the curve (AUC) using the software Graphpad Prism.

RESULTS

Animals. At the time of killing, 5-wk-old TN ducklings were heavier than CA birds (1.74 ± 0.05 kg in TN vs. 1.4 ± 0.08 kg in CA, P < 0.05), despite a lower food intake (7). Relative (per unit body mass) liver mass was higher in CA than in TN birds (3.5 ± 0.06 vs. 3.0 ± 0.12%, P < 0.001).

Incorporation into FA with incubated hepatocytes using [1-14C]acetate. Incorporation was expressed in nanomoles 14C incorporated per milligram dry weight cells after 30, 60, and 90 min of incubation. Figure 1A shows that basal incorporation of 14C into FA was linearly related to incubation duration in both TN (R2 = 0.98) and CA (R2 = 0.99) ducklings. The basal rate was significantly higher in CA than in TN ducklings after 60 min (+110%, F = 7.0, P < 0.05) and 90 min of incubation (+126%, F = 12.0, P < 0.01). To assess global effects during the whole duration of incubation, AUC was calculated (Fig. 1B). Global incorporation was much higher (P < 0.05) in CA than in TN ducklings in all conditions, basal (+115%), with 100 nM insulin (+144%) or 1 nM glucagon (+168%). Insulin increased (+11%, F = 7.6, P < 0.05) whereas glucagon decreased (~22%, F = 7.8, P < 0.05) global 14C incorporation in CA ducklings.
Basal oxidation was much higher in CA than in TN ducklings after 60 min (+164%, $F = 14.3$, $P < 0.05$) and 90 min (+216%, $F = 15.7$, $P < 0.05$) of incubation. Global glucose oxidation rate (AUC, Fig. 4B) was higher in CA than in TN ducklings both in basal conditions (+171%, $F = 13.9$, $P < 0.05$) and in the presence of insulin (+173%, $F = 12.8$, $P < 0.05$) but not in the presence of glucagon ($P > 0.05$). In CA ducklings, insulin increased (+14%, $F = 17.1$, $P < 0.05$) whereas glucagon decreased (−51%, $F = 8.7$, $P < 0.05$) glucose oxidation (Fig. 4B).

[1-14C]Oleate oxidation rate of tissue homogenates. In both liver and skeletal muscles, mitochondrial oxidation accounted for the majority of total oxidation of [1-14C]oleic acid (Fig. 5). The contribution of peroxysomes to total oxidation was 19% in liver and 9–10% in muscle homogenates from both TN and CA ducklings.

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**Fig. 2.** Incorporation of $^{14}$C from [U-14C]glucose into fatty acids in hepatocytes from TN (circles; $n = 3$) or CA (squares; $n = 5$) ducklings. A: basal incorporation in absence of hormonal stimulation is presented as a function of the duration of incubation. B: total [U-14C]glucose incorporation during 90 min of incubation (area under the curve) was calculated in basal conditions and in response to $100$ nM insulin or $1$ nM glucagon in TN (open bars; $n = 3$) and CA (filled bars; $n = 5$) ducklings. Data are expressed as means ± SE. Within a group of ducklings (TN or CA), values with different letters were significantly different ($P < 0.05$). ★$P < 0.05$ vs. TN controls.

0.05) in the presence of insulin (+341%, $F = 15.6$, $P < 0.05$) and glucagon (+158%, $F = 14.7$, $P < 0.05$). Insulin did not significantly affect incorporation in TN and CA birds, whereas glucagon decreased it in CA ducklings (−51%, $P < 0.05$; Fig. 2B).

**Enzyme activity.** Activity of key enzyme of lipogenesis was measured in liver homogenates from TN and CA ducklings (Fig. 3). Activities of G6PDH and ME expressed per milligram protein (specific activity) were higher in liver from CA than from TN ducklings (+30 and +35% for G6PDH and ME, respectively, $P < 0.05$). FAS activity was low and did not differ between groups ($P = 0.3$) (Fig. 3A), whereas ACCx-specific activity was higher in liver homogenates from CA than from TN ducklings (+70%, $F = 5$, $P < 0.05$). When expressed per unit body mass (Fig. 3B), to take into account changes in relative liver mass, the capacity of the four lipogenic enzymes was increased (+50 to +100%, $P < 0.05$) by cold acclimation.

[U-14C]glucose oxidation by hepatocytes. Basal glucose oxidation rate of incubated hepatocytes, estimated by the amount of $^{14}$CO$_2$ released (Fig. 4A), increased linearly with the duration of incubation ($R^2 = 0.99$ in TN and CA ducklings).
Gastrocnemius muscles exhibited lower total and mitochondrial oleate oxidation rates than the liver. As expected, the red part of the muscle showed a higher oxidation rate than the white part. In all tissues, total oleate oxidation rate was higher in CA than in TN ($P < 0.05$) due to an increase in mitochondrial oxidation rate ($61, 29, \text{ and } 81\% \text{ in liver, red and white gastrocnemius, respectively, } P < 0.05$).

**DISCUSSION**

**General findings.** The present study provided for the first time direct insights into the acute control of lipogenesis in isolated duckling hepatocytes and into the adaptive changes induced by cold acclimation. Two main results emerged from this study. First, de novo FA synthesis was high in duckling hepatocytes compared with other species and was further increased after cold acclimation in association with a slightly higher stimulatory effect of insulin and much higher activities of hepatic lipogenic enzymes. Second, CA ducklings showed higher tissue ability to oxidize lipids, further supporting the central role of lipids in avian cold acclimation.

High activity of de novo lipogenesis in duckling hepatocytes. Lipogenesis was measured by the in vitro incorporation of [1-14C]acetate or [U-14C]glucose into the lipid fraction mainly corresponding to FA (35, 36). With both precursors, the constant rate of lipogenesis in vitro assessed by linear incorporation (Figs. 1 and 2) ensured the suitability of our in vitro preparation. Duckling hepatocytes incorporated 14C from acetate or glucose into FA at high rates compared with other species (Table 1). The rate of incorporation of a given precursor depends on its concentration, its dilution into intermediary pools, and cellular partitioning of substrates. Depending on these parameters, hepatocytes from ad libitum-fed rats usually incorporate acetate at a higher rate than glucose (Table 1), but when hepatocytes are obtained from refed animals, this differential incorporation disappears and incorporation rates are higher (Ref. 14; Table 1). Results in ad libitum-fed ducklings therefore resemble those obtained in this unusual refeeding situation in rats and underline that our data are in the higher range of reported values of hepatocyte lipogenesis.

A high rate of incorporation from glucose is not unusual in birds. Quail hepatocytes, for instance, were

FIG. 4. Glucose oxidation (incorporation of 14C from [U-14C]glucose into CO$_2$) by hepatocytes from TN (●; $n = 3$) or CA (▲; $n = 5$) ducklings. A: basal incorporation in absence of hormonal stimulation is presented as a function of the duration of incubation. B: global 14CO$_2$ production during 90 min of incubation (area under the curve) in basal conditions and in response to 100 nM insulin or 1 nM glucagon was calculated in TN (open bars; $n = 3$) and CA (filled bars; $n = 5$) ducklings. Data are expressed as means ± SE. Within a group of ducklings (TN or CA), values with different letters were significantly different ($P < 0.05$). ▲$P < 0.05$ vs. TN controls.

FIG. 5. Total and mitochondrial [1-14C]oleate oxidation rate of tissue homogenates from TN (open bars; $n = 7$) or CA (filled bars; $n = 5$) ducklings. Oxidation rates were determined in liver (A), red (B) and white (C) gastrocnemius muscles. Oxidation rates are expressed as nanomoles of oxidized oleate per minute per gram of tissue. Data are expressed as means ± SE. ▲$P < 0.05$ vs. TN controls.
shown to extensively use glucose for de novo lipogenesis (35), but the incorporation rate of 14C from glucose in duckling hepatocytes was even higher (2-fold) (Table 1). The high rate of glucose incorporation into FA of duckling hepatocytes occurred in parallel with high activities of key-limiting enzymes of lipogenesis. FAS activity of duckling liver, for instance, was 10-fold higher than that in chicken (27). Although high, enzyme activities in duckling liver were much lower (60-fold) than in liver from force-fed geese (25). These observations lead us to speculate that the high incorporation rate of 14C from glucose into FA and high level of lipogenic enzymes may be a characteristic of avian species susceptible to hepatic steatosis such as muscovy ducks (Cairina moschata) or Landes geese (Anser anser) (30), but the molecular basis of such characteristic is unclear and remains to be determined.

### Increased basal lipogenesis and acute hormonal control after cold acclimation

Present results clearly showed that the in vitro incorporation of acetate (+115%; Fig. 1B) and glucose (+194%; Fig. 2B) into FA was much higher in CA than in TN duckling hepatocytes. These results indicate a marked stimulation of de novo hepatic lipogenesis after cold acclimation. Similarly, de novo FA synthesis in vivo was increased in response to cold acclimation in rat tissues including liver, brown and white adipose tissues (21, 44).

The cold-induced activation of lipogenic activity of duckling-isolated hepatocytes was associated with a slightly higher stimulatory effect of insulin (+11% in CA ducklings), whereas hepatocytes from TN ducklings were unresponsive. This rather low responsiveness in hepatocytes isolated from ducklings in a postprandial state may likely be related to prior in vivo stimulation by endogenous release of insulin. The cold-induced rise in hepatocyte responsiveness to insulin in ducklings compares with that described in cold-acclimating rats in vivo (16) and may possibly involve a higher density of membrane receptors and/or enhanced postreceptor mechanisms.

Glucagon clearly inhibited lipogenesis in CA ducklings (−22 and −51% with acetate and glucose, respectively) as in other species (15, 36). Glucagon, which was proposed as a potential mediator of avian cold-induced nonshivering thermogenesis by stimulating thermogenic processes (4, review in Ref. 18) through increased lipolysis in adipose tissue (7), cannot be responsible for the cold-induced changes in lipogenesis occurring in duckling liver. This suggests that cold acclimation may rather be under a coordinated control by several hormones (18). Insulin, for instance, may play an important role, and an increased stimulatory effect of insulin on glucose transport was already noted in skeletal muscle from CA ducklings (43). Thus, the global higher sensitivity to insulin after cold acclimation may favor both hepatic lipogenesis and skeletal muscle uptake of substrates.

### Increased hepatic activity of key lipogenic enzyme after cold acclimation

Cold acclimation increased hepatic ACCx activity (+70%), which can contribute to the increased basal incorporation of acetate into FA in hepatocytes from CA ducklings (+115%; Fig. 1B). ACCx activity was already proposed as a prior index of lipogenesis in birds on account of the strong correlation between its activity and FA synthesis in chicken hepatocytes (29). In cytoplasmic lipogenic process, the product of ACCx is the substrate of the FAS enzyme, and the lack of marked change in FAS activity after cold acclimation suggests that FAS activity may not be limiting. However, when expressed per unit body weight to take into account the change in relative liver mass, both ACCx and FAS capacity were increased in CA ducklings (+100 and +52%, respectively). This way of expressing the data may more adequately reflect the actual global changes in lipogenic capacity of CA ducklings. On the other hand, cytoplasmic supply of NADPH by the activity of ME, another key enzyme of lipogenesis in birds (24), was also increased in hepatocytes from CA ducklings (+35%).

Interestingly, the activity of G6PDH, the key enzyme of the pentose-phosphate cycle, which also produces NADPH for lipogenesis, was much higher in ducklings than usually observed in pigeon or chicken liver (46). In those latter species, ME was found to be 50- to 100-fold more active than G6PDH (42, 46), leading to the idea that in avian liver, most NADPH comes from ME. By contrast, the relative contribution of the two pathways generating NADPH (through ME or G6PDH) was estimated to be ~50% in rat liver (review in Ref. 26). These data indicate that the pentose-phosphate pathway may function in ducklings, contrary to what is usually assumed in birds (35), and may contribute to the use of glucose by duckling hepatocytes. It may be particularly important during metabolic adaptation to

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**Table 1. Comparison of incorporation of labeled substrates into fatty acids using isolated hepatocytes from different species**

<table>
<thead>
<tr>
<th>Species</th>
<th>Incorporation Rate</th>
<th>Reference</th>
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<tbody>
<tr>
<td>Rat hepatocytes</td>
<td></td>
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<tr>
<td>[1-14C]acetate</td>
<td>1 mM</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>5 mM</td>
<td>8</td>
</tr>
<tr>
<td>[U-14C]acetate</td>
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<td>19</td>
</tr>
<tr>
<td></td>
<td>20 mM</td>
<td>63</td>
</tr>
<tr>
<td>[U-14C]glucose</td>
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<td>5</td>
</tr>
<tr>
<td></td>
<td>20 mM</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>20 mM</td>
<td>56</td>
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<table>
<thead>
<tr>
<th>Bird hepatocytes</th>
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<tbody>
<tr>
<td>[1-14C]acetate</td>
<td>2.5 mM</td>
<td>10</td>
</tr>
<tr>
<td>chicken</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TN duckling</td>
<td>2.5 mM</td>
<td>59</td>
</tr>
<tr>
<td>CA duckling</td>
<td>2.5 mM</td>
<td>124</td>
</tr>
<tr>
<td>[U-14C]glucose</td>
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<td>38</td>
</tr>
<tr>
<td>quail</td>
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<td></td>
</tr>
<tr>
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<td>79</td>
</tr>
<tr>
<td>CA duckling</td>
<td>10 mM</td>
<td>170</td>
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Values from other studies were recalculated assuming that 1 mg dry wt is equivalent to nearly 5 × 10^5 cells, that the wet-to-dry mass ratio is 3.75, and that there is 0.2 g protein/g hepatocyte (8, 14). Refed rats had a limited access to feed (3 h/day) and were killed 4–7 h after refeeding. The concentration of precursor is given. TN, thymoneutral; CA, cold acclimated.
cold as the activity of G6PDH is further increased in CA ducklings.

Increased enzyme activity, such as ACCx and FAS, in the cold indicates long-term adaptation of lipogenesis that may depend on a control by insulin and thyroid hormones. One cannot exclude the possibility that part of the effect of cold is also related to differences in food intake as lipogenesis is modulated by the nutritional status of the animal. For instance, increasing carbohydrate intake in growing chickens resulted in increased hepatic ACCx and FAS activities. The higher food intake of 5-wk-old CA ducklings (+30%; Ref. 7) may thus contribute to the increased de novo lipogenesis after cold acclimation but can hardly explain the entire stimulation (+115 to +194%).

Increased glucose oxidation by hepatocytes after cold acclimation. Basal and insulin-stimulated oxidation rates of glucose were much higher (~70%; Fig. 4B) in hepatocytes from CA than from TN ducklings in connection with higher rates of de novo lipogenesis. The increased ATP need of a stimulated lipogenesis may likely contribute to this phenomenon. The ratio of glucose oxidation to glucose incorporation (14C into CO2/14C into FA) was lower in duckling hepatocytes (ratio ~2) than in quail hepatocytes (ratio ~7; Ref. 35) or turkey liver slices (ratio ~5; Ref. 37). Such observation may suggest that glucose may be preferentially orientated to de novo lipogenesis rather than to oxidation in hepatocytes from avian species susceptible to liver steatosis, such as muscovy ducklings.

The inhibitory effect of glucagon on glucose oxidation (~51%) in CA hepatocytes probably depends on a stimulation of neoglucogenesis vs. glycolysis (36). An increased neoglucogenic effect of glucagon was previously reported in perfused liver from CA ducklings (6). In avian hepatocytes, glucagon is known to inhibit glycolysis at the level of phosphofructokinase, thus decreasing glycolytic flux into pyruvate, which in turn results in lower cytosolic citrate concentration and thus decreased ACCx activity (review in Ref. 45).

Increased liver and skeletal muscle rate of oleate oxidation in CA ducklings. The question arises as to the fate of the FA synthesized in the liver. Because CA ducklings are not fattier than TN controls, we assumed that FA might be more oxidized in thermogenic tissues and measured the in vitro potential for oleate oxidation. In duckling tissues, total oleate oxidation capacity was higher in liver than in skeletal muscles and higher in red than in white skeletal muscle, in agreement with other studies in rats or bovines (32). Total oxidation of oleate mainly depended on mitochondrial oxidation with a low contribution of peroxysomal oxidation (19% in liver and 10% in skeletal muscles) in agreement with data in rats (32). In liver, the mitochondrial potential for FA oxidation was higher in CA than in TN ducklings (+61%) in keeping with the increased hepatic cytochrome oxidase activity (+54%) measured in these birds (2). Skeletal muscles also showed an increased potential for oleate oxidation, especially in white muscles endowed of the lowest initial capacity. Part of this effect can be related to the higher proportion of oxidative muscle fibers in CA ducklings (review in Ref. 18) to their higher cytochrome oxidase activity and mitochondrial content (2, 38). Increased potential for oleate oxidation is also consistent with the enhanced muscle capacity of intracellular transport by FA-binding proteins in CA ducklings (7) and with the increased muscle expression of an avian uncoupling protein (34). The potential role of this avian uncoupling protein in lipid oxidation and thermogenesis clearly deserves further investigation.

Physiological implication of increased hepatic lipogenesis in CA ducklings. One can reasonably assume that hepatic lipogenesis is increased in the cold to fuel higher energy expenditure for thermoregulatory purpose. Indeed, despite higher lipogenic activity, CA ducklings are not fattier and show increased heat production at their rearing temperature by the development of muscle-nonshivering thermogenesis (3, 17). Lipids may play a peculiar role in cold-induced avian muscle-nonshivering thermogenesis both as activators (38, review in Ref. 18) and as energetic substrates. An increased lipogenic activity of the liver can therefore be of adaptive value consistent with an increased skeletal muscle FA oxidation capacity. FA supply to skeletal muscle oxidation may originate both from adipose tissue lipolysis that is stimulated in CA ducklings (7) and from enhanced hepatic lipogenesis (present study). Liver mainly exports lipids under the form of triglycerides forming very low-density lipoproteins that may be taken up by skeletal muscle through an enhanced lipoprotein lipase activity in CA ducklings (7).

The increased lipogenic activity of duckling hepatocytes after cold acclimation emphasizes the crucial need of lipids for regulatory thermogenesis and may therefore represent a key step enabling survival under harsh energetic conditions. Similarly, in rats, BAT lipogenesis was increased together with thermogenic activity during cold acclimation (21, 44), whereas it was decreased together with thermogenic capacity following a high-protein, carbohydrate-free diet (11).

Conclusion. In conclusion, the present study addressed for the first time the acute control of lipogenesis in isolated duckling hepatocytes and into the adaptive changes induced by cold acclimation. Present data underline the high lipogenic capacity of ducking hepatocytes and show that this capacity can be further increased in the cold in relation with a higher stimulatory effect of insulin and higher activities of lipogenic enzymes. Hepatic lipogenesis may fuel thermogenic tissues endowed with higher capacity to oxidize FA and/or contribute to fat store replenishment. Adaptive changes in hepatic lipogenesis may thus represent an important step in the metabolic adaptation to cold of growing ducklings.

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