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ABSTRACT

Rapid growth is of crucial importance for Adélie penguin chicks reared during the short Antarctic summer. It partly depends on the rapid ontogenesis of fat stores that are virtually null at hatching but then develop considerably (x 40) within a month to constitute both an isolative layer against cold and an energy store to fuel thermogenic and growth processes.

The present study aimed at identifying by RT-PCR the major transcriptional events that chronologically underlie the morphological transformation of adipocyte precursors into mature adipocytes from hatching to 30 days of age.

The peak expression of GATA3, a marker of preadipocytes, at day 7 post-hatch indicates a key proliferation step possibly in relation with the expression of C/EBPα. High plasma total T_3 levels and high levels of GH receptor transcripts at hatching suggested that GH and T_3 play early activating roles to favour proliferation of preadipocyte precursors. Differentiation and growth of preadipocytes may occur around day 15 in connection with increased abundance of transcripts encoding IGFI, PPARγ and C/EBPβ gradually leading to functional maturation of metabolic features of adipocytes including lipid uptake and storage (lipoprotein lipase, fatty-acid synthase) and late endocrine functions (adiponectin) by day 30.

Present results show a close correlation between adipose tissue development and chick biology and a difference in the scheduled expression of regulatory factors controlling adipogenesis as compared with *in vitro* studies using cell lines emphasizing the importance of *in vivo* approaches.

INTRODUCTION

Efficient endothermy, that is restricted to mammals and birds, has played a central role in the conquest of cold environments by vertebrates. This elaborate physiological function requires both an integrative system of sensing and regulating body temperature and a powerful tachymetabolism. It also necessitates an efficient isolative layer to reduce the energetic cost of maintaining body temperature at a high level, independently of ambient temperature, that may otherwise represent an excessive part of energy metabolism for endotherms of Polar Regions.

Living below 0°C indeed generates tremendous constraints not only to adult organisms such as penguins but also to newborns that exhibit a high surface to volume ratio, a poor insulation and a low capacity to generate heat (11). Newborn penguins are thus facing an inevitable dilemma between collecting as much energy as possible to rapidly mature in time and allocating energy for thermoregulatory purpose. Solutions can be found in parental thermal protection and rapid building up of efficient insulation and thermoregulatory capacity (11). Adipose tissue may contribute to both last aspects as an isolative layer protecting against cold exposure and as an energy reserve to fuel growing and/or thermogenic tissues. Rapid ontogeny of adipose tissue stores may thus be of major importance for survival of young penguins, but this implies that part of food energy must be stored as adipose tissue to the detriment of the somatic growth of the body.

The case of Adelie penguin, that can be considered as a long-lived species (predicted maximum lifespan ~ 24 years) (23), is of particular interest. Adults weighing approximately 4.6 kg (23) breed in Antarctica during the summer season. The nest is made of small size stones offering a poor thermal protection against climatic hazards. Eggs are therefore incubated under the brood pouch and maintained warm by the adults. After hatching, the thermal protection offered by adults is limited to the first days of life, until chicks are able to reach the incubation pouch (between 7-15 days). The thermoregulatory ability of newborns is gradually enhanced as chick energy expenditure increases in the nestling phase (age 0-11 days) and then stabilizes during the crèche phase (14-40 days) (7). Because summer period is short, newborns have about eight weeks to reach a critical size

and moult before departure to sea, to get nutritional emancipation. Because of the high thermal conductance of water and the very low temperature of polar seas, powerful isolation by feathers and subcutaneous fat pads is essential at that stage.

Accumulating fat reserves depends on adipocyte differentiation, proliferation and lipid synthesis. The biological process of adipogenesis has been extensively studied *in vitro* using a number of preadipocyte cell lines (20, 30, 39, 12, 25, 30). These studies have identified pro- and anti-adipogenic transcription factors and established that the differentiation process is the result of a subtle equilibrium between these different actors. Two transcription factor families have emerged as the key determinants of terminal adipocyte differentiation: the peroxisome proliferator-activated receptor γ (PPARγ) and the CCAAT/ enhancer-binding proteins (C/EBPα and β). Experimental studies performed *in vitro* and *in vivo* support the view that these factors act in a coordinated and sequential manner to control the various steps of adipogenesis (37, 29). In birds most studies have investigated the lipogenic genes in chickens because fatness is of primary importance in poultry breeding (2, 31). By contrast, our knowledge on adipose tissue development in wild, cold-adapted species is very limited despite the eco-physiological importance of this process. Elucidating the mechanisms of adipose tissue development in a context of massive energy constraints because of cold may also contribute to enlighten fundamental key regulatory steps.

The aim of the present study was to delineate the developmental phases of adipose tissue in Adelie penguin chicks from hatching to the first month of life, a critical period of tissue maturation leading to thermal emancipation. For that purpose, we measured the mRNA expression of the main adipogenic transcriptional factors, the growth factors known to be involved in adipogenesis and the functional markers of terminal differentiation that lead to the phenotype of mature adipocytes. The molecular events identified will be discussed in the context of growing chicks and correlated with plasma fuel concentrations.

MATERIALS AND METHODS

Ethical approval for all procedures was granted by the ethics committee of the French Polar Research Institute and by the Ministère de l'Environnement. Our experiments conformed to the Code of Ethics of Animal Experimentation in the Antarctic.

Animals

The study was conducted at Dumont d'Urville, Adélie Land (66°07'S – 140°00'E) in Antarctica and more precisely on Pointe Geologie archipelago where about 34,000 pairs of Adelie penguins (*Pygoscelis adeliae*) nest every year (18). Tissue and blood samples were collected during two successive summer seasons in 2005-2006 and 2006-2007. Adelie penguins generally lay two eggs and the smallest chick does not usually survive. To minimize impact of our study on breeding success, we only used second chicks in the present study. Following our program authorization, 16 Adelie chicks were euthanized each year (4 chicks for each point of growth: hatching, 7, 15 and 30 days old) after fluothane anaesthesia. All chicks were weighed before organs, muscles, subcutaneous and retro-peritoneal adipose tissues were quickly excised, weighed and immediately frozen in liquid nitrogen. All samples were stored at -80°C until RNA extraction.

Climatic conditions

The breeding season of Adelie penguins begins at the end of October. After a period of nest building and courtship, two eggs are generally laid by the females (1.8 ± 0.4) (1). The eggs hatch early in December after an incubation period of 32-38 days. Then, chick growth depends exclusively on parental feeding up to the age of 2 months when departure to sea occurs. Given the brevity of the Antarctic summer at high latitude, climatic changes and their impact on the abundance and location of marine resources largely influence the survival and growth of the chicks. It might be supposed that harsh climatic conditions impact biological responses during the first

stages of their development. We have therefore collected the daily measurements of air temperature (°C) to appreciate the environmental conditions during chick growth.

Plasma parameters

Total blood (0.5 to 1 mL) was collected on heparin at the time of sacrifice. All samples were centrifuged at 5,000 *g* for 10 min. Triglycerides (PAP, BioMérieux, Marcy L'Etoile, France) and non-esterified fatty acids (NEFA, NEFA-C, Wako Chemicals GmbH, Neuss, Germany) were assayed using commercially available kits according to the manufacturer's recommendations. Plasma glucose was assayed using a glucometer (Accu-Check, Roche, Meylan, France). Plasma total 3, 5, 3'- triiodo-L-thyronine (T_3) was measured using an ELISA kit (Calbiotech INC., Spring Valley, CA USA).

Histological analysis of adipose tissue

Samples of subcutaneous adipose tissue from 1, 7, 15 and 30 days old chicks, located in ventral position along the pectoralis muscle, were immediately fixed in 4% paraformaldehyde, dehydrated and embedded in paraffin. Sections of 7 µm thick were stained by HES (Haemalum, Eosin and Saffron) for histological evaluation.

Quantification of mRNA expression by semi-quantitative RT-PCR

RT-PCRs were performed in the biology laboratory of Adelie Land (Biomar) to avoid mRNA damage during the long trip back to France. Total RNA were extracted from frozen subcutaneous adipose tissue of Adelie chicks. Tissues were homogenized in Trizol-Reagent (Invitrogen - Life Technologies, Cergy Pontoise, France) and total RNA isolated according to the manufacturer's protocol. Reverse transcription (RT) of 1 µg RNA was performed using 200 U of M-MLV - Reverse transcriptase (Promega, Charbonnières les Bains, France). The cDNAs obtained were submitted to polymerase chain reaction (PCR) amplification using 2.5 U of *Taq* DNA polymerase (Eurobio, Les Ullis, France) and 1 µM of forward and reverse primers (Invitrogen - Life

Technologies, Cergy Pontoise, France). The primers used were defined according to chicken (*Gallus gallus*) gene sequences and are listed in Table 1.

The amplified products were separated on agarose gels and band intensity was quantified with a Kodak Digital Science TMID image analyse software. For each gene, band intensity was normalized against β-actin, a housekeeping gene that shows little change in adipose tissue of growing Adelie penguin chicks. PCR amplification of actin was performed using the same RT reaction as the target gene. In all PCR reactions, care was taken to use the appropriate number of amplification cycles to remain in the exponential phase of the amplification process and avoid saturation. Separate duplicate PCR reactions were used to verify the expression profile of each gene during chick growth. The effect of age was analysed on samples obtained within the same PCR run and separated on the same gel containing samples from birds at different ages. Results from duplicated reactions were averaged and used for quantification.

Sequencing

The required amount of material was amplified by PCR with a "High fidelity" *Taq* DNA polymerase (Invitrogen - Life technologies, Cergy Pontoise, France) and was sequenced (Genoscreen, Lille, France) to confirm the specific amplification of the targeted cDNAs and transcripts.

Statistical analysis

Data are expressed as means ± S D. Statistical differences have been evaluated with a two-way ANOVA using StatView program of the MacIntosh system. A Fisher protected least-significant difference (PLSD) post-hoc test was used for group comparisons. A p value < 0.05 was considered as statistically significant.

RESULTS

Body mass and morphological data

Figure 1A gives a general survey of living conditions of Adelie penguin chicks. After birth, chicks are fully protected against climatic hazards by their parents. That protection is efficient as long as the body size of the chicks allows them to completely enter the brood pouch of the parent. Then, chicks are progressively exposed to harsh climatic conditions from fifteen to thirty days old, a critical period during which they must ensure their thermal autonomy, related in part to the amount of subcutaneous fat layer. We thus choose four different ages to study the development of subcutaneous white adipose tissue: hatching time (D1), day 7 (D7), characterized by optimal parental thermal protection, day 15 (D15), that correspond to partial exposure to ambient conditions and day 30 (D30) when chicks are continuously exposed to cold environment. During their first month of life, the mean daily temperature remained below freezing (Figure 1A). Despite the harsh climatic conditions, chick growth was remarkably rapid as body weight increased more than twenty fold within the first month of life showing that at least during that period, even the second chick is well fed (Figure 1A). During that period, the mass of adipose tissue (subcutaneous plus retroperitoneal) increased rapidly. It was multiplied by 40 over the first month of life (Figure 1B). Such increase in fat mass may likely imply both proliferation of adipose stem cells, differentiation into adipose cells and hypertrophy of pre-existing adipocytes. A close correlation has been found between subcutaneous and retroperitoneal adipose tissue mass (Fig. 1C). Subcutaneous adipose tissue was studied throughout the study on account of its higher abundance than retroperitoneal adipose tissue (18) and its greater role in thermal insulation.

Gene expression profile in adipose tissue from hatching to day 30 of development

To understand the molecular basis underlying adipogenesis, we first analysed the expression of pro and anti-adipogenic transcription factors known to be involved in adipocyte differentiation. The relative abundance of PPARγ mRNA markedly increased at D15 of growth and remained high

until D30 (Figure 2). The same pattern was observed for the mRNA encoding C/EBP□ (Figure 2). C/EBPα, a potent enhancer of adipocyte terminal differentiation, known to be expressed in parallel to PPARγ in 3T3-L1 cells, reached maximum levels at D7 and remained unchanged until D30 (Figure 2). The GATA binding protein 3, a member of the GATA family of transcription factors involved in adipocyte developmental processes and characterizing preadipocytes, was specifically expressed at D7 and down regulated to basal levels thereafter (Figure 2).

We also investigated potential growth factors known to stimulate adipogenesis (Figure 3). Because of the difficulty to determine the concentrations of circulating hormones in Adelie penguins, we used an indirect approach by measuring the expression of their receptors in adipose tissue. We first investigated the mRNA abundance of insulin-like growth factor I receptor (IGF-1R) as insulin may likely promote differentiation through these receptors (20). There was a significant increase in IGF-1R mRNA relative abundance at D15 and D30 compared with D1 and D7 (Figure 3). This increase occurred in parallel with a peak of IGF-1 mRNA at D15 (Figure 3) suggesting a possible auto- / paracrine action of fat-derived growth factors in the adipogenesis of Adelie chicks. As T_3 (3, 5, 3'-triiodo-L-thyronine) and GH (growth hormone) are essential for growth, differentiation and maintenance of metabolic homeostasis, we explored the expression levels of $T_3R\alpha$ and GHR in adipose tissue of Adelie chicks. $T_3R\alpha$ mRNA relative abundance was already high at hatching but despite trends, there was no significant post-hatching change in expression (Figure 3). The expression profile of mRNA encoding GHR was more variable as it was increased at D1 and D15 compared with D7 and D30 (Figure 3) suggesting a peculiar role of GH at these specific ages.

Finally, we studied the expression of genes encoding proteins characteristic of adipose cell differentiation including FAS (Fatty Acid Synthase), LPL (Lipoprotein Lipase), FABP (Fatty Acid Binding Protein) and adiponectin (Figure 4). FAS and LPL mRNA relative abundances markedly increased at D15 and D30 while FABP reached maximal levels at D30 (Figure 4). The expression of mRNA encoding adiponectin gradually increased between D7 and D30 (Figure 4).

Penguin / chicken / mouse / gene similarities

The sequences obtained for each Adelie penguin cDNA studied were compared to the corresponding chicken and mouse gene sequences and the degree of similarity is summarized in Table 2. Sequence similarities were found between 81-95% with chicken corresponding sequences confirming the specificity of the amplifications.

Plasma substrate levels

As shown in Table 3, plasma NEFA (non-esterified fatty acids) concentration was not modified whatever the age while plasma triglycerides and glucose increased from D1 ($p < 0.05$). From D7 to D30 plasma triglycerides and glucose remained stable. Plasma T_3 concentration slightly decreased from hatching to D30 with a significant effect of age observed at D30 ($p < 0.05$).

DISCUSSION

This study has identified for the first time the molecular events potentially involved in adipose tissue development during the first month of life of Adelie penguins.

Rapid development of adipose tissue involves adipogenic genes

Changes in tissue weight indicate that adipose tissue develops rapidly during the posthatching growing period. There is little doubt that adipose tissue growth results from both an increase in cell number and cell size. Indeed, as shown in figure 5, adipose cell size significantly increased during the first month of life. The rapid growth of fat stores until day 15 (Fig 1B), when chicks benefited from the maximal thermal protection by their parents, suggests that adipose tissue development is favoured by chick intense feeding and energy saving allowed by parental care. It follows that adipose tissue development precedes the marked cold exposure that occurs after thermal emancipation *ie* after D15.

Factors responsible for adipogenesis have never been studied in wild birds. Much of our knowledge on the transcription control of adipogenesis comes from studies of cultured mouse 3T3-L1 (38, 28) and from a few *in vitro* studies using cultured chicken preadipocytes (9, 15, 25). These studies generally state that the early expression of C/EBPβ and δ triggers the expression of C/EBPα and PPARγ which co-ordinately activate the transcription of genes that rise to the mature adipocyte phenotype. Our results point out a striking difference with this general consensus sequence of promoter activation. Indeed, we found a precocious activation of C/EBPα (D7, Figure 2) preceding that of PPARγ and C/EBPβ (D15, Figure 2). This observation in Adelie penguin *in vivo* differs from the *in vitro* differentiation of cultured chicken preadipocytes showing that PPARγ mRNA expression level is rapidly increased before C/EBPα and C/EBPβ gene activation (25). It is not clear as to whether this relates to differences between *in vivo vs. in vitro* situations and/or species differences but a precocious activation of C/EBPα preceding that of other regulating proteins was also reported during pig foetal development without the expression of C/EBPβ and δ (17, 22).

Although C/EBPα expression alone is not sufficient to induce adipocyte development in culture (17), it may represent a necessary step that is conserved during evolution and found in birds and mammals.

Nevertheless, present data also underline a marked up-regulation of PPARγ in parallel with the final maturation of adipocytes. It is also consistent with the observation that PPARγ is not expressed or is expressed at low levels in preadipocytes and is turned on during differentiation, prior to the expression of most adipocyte genes, many of which containing PPAR-binding sites (reviewed in 35, 3). This is in keeping with the observation that PPARγ plays an important role in the regulation of fat deposition (31) and the recent demonstration that transient transfection with siPPARγ inhibits the differentiation of chicken preadipocytes (41). Nevertheless, present data are strengthening the notion that although PPARγ may be sufficient to trigger the adipogenic program, C/EBPα is required for many aspects of adipocyte differentiation and maturation (16).

To counteract the action of transcription factors that promote adipogenesis, members of the GATA-binding family, which are zinc-finger DNA-binding proteins involved in developmental processes, rather act as adipogenic repressors. Previous studies indicated that GATA3 is expressed in preadipocytes and down-regulated during adipocyte differentiation (38). Accordingly, we found a high abundance of GATA3 mRNA early at D7 that may correspond to an early proliferation of new preadipocytes. The return of mRNA encoding GATA3 back to basal level after D7 suggests that preadipocytes have then been engaged into adipocyte differentiation.

Implication of endocrine factors during adipogenesis

Adipocyte differentiation can be induced by several factors such as IGF-1, GH and T_3 and (40, 26, 12) acting on specific receptors expressed by adipocytes.

Present results indicate an increased abundance of mRNA encoding IGF-1R, IGF-1 and GHR at D15. Such time-related change in expression, at least at the mRNA level, is consistent with adipose cell differentiation at that stage (Figures 3, 5). The marked upregulation of IGF-1 by D15 is

in accordance with a major role of the peptide as a regulator of cell proliferation regulating adipose tissue growth and differentiation of preadipocytes into adipocytes (6). Interestingly, the concomitant expression of IGF-1 and IGF-1R observed at D15 suggests an additional autocrine / paracrine effect of the peptide. The correlation between the changes in IGF-1 and GHR mRNA favours a causal link consistent with the known GH-activated expression and secretion of IGF-1 in preadipocytes (14). The activity of GH may thus result from both a direct action of the hormone on its receptors and an indirect action mediated by IGF-1. On account of the stimulatory role of GH on the pool of adipocyte precursor cells capable of differentiating into mature adipocytes (6), it is postulated that the high level of GHR expression found at hatching contributes to an early phase of proliferation occurring during the first week posthatching. Altogether, present results emphasize that the proliferation, differentiation and metabolism of adipose tissue may be highly regulated by the GH/IGF-1 system in Adelie penguins.

Thyroid hormone (T_3) is also critical for the growth and differentiation of a number of tissues including adipose tissue. The high levels of circulating T_3 after hatching may contribute to early activation of adipose tissue development. Thereafter, the lowering of plasma T_3 levels after day 15 is likely to reduce metabolic rate and thus spare energy substrates that would be available for storage in adipose tissue. Most T_3 effects are mediated by interactions with nuclear T_3 receptors (T_3R), which are ligand-dependent transcriptional factors that positively or negatively regulate T_3 responsive genes (4). In birds, cDNAs encoding at least two T_3Rs (α and β) have been isolated in chickens (33, 36) and ducks (21). A recent study (43) demonstrating that a knock-in mutation in the TRα isoform reduced adipogenesis and PPARγ expression, prompted us to explore TRα expression during the adipogenesis of Adelie chicks. Present results indicated high but rather constant relative abundance of TRα mRNA from D1 to D30 (Figure 3). This *in vivo* observation is in keeping with *in vitro* findings indicating that during the adipogenesis of 3T3-L1, TRα is constitutively expressed in preadipocytes as well as in mature adipocytes (43).

Late expression of functional markers of matured adipocytes

The apparition of functional markers of maturation such as LPL, FAS from D15 and FABP and adiponectin from D30 (Figure 4) finalizes the program of adipogenesis in the Adelie Chicks. Indeed, LPL that is responsible for the hydrolysis of circulating triglycerides into free fatty acids and glycerol, is known to be an early marker of adipose cell maturation and will contribute to the storage of circulating lipids arising from food intake rich in lipids or endogenous lipogenesis. Despite the fact that much of the regulation of the LPL occurs at the post-transcriptional level (27, 10), present results clearly indicate a surge in the relative abundance of LPL mRNA at that point of adipose tissue development. This is in agreement with previous observations of increased LPL gene transcription during adipogenesis in rat (28) and 3T3-L1 adipocytes (32). Similarly, transcription of the LPL gene in the heart increased 10-fold in rat pups (34). Although *de novo* lipogenesis in birds mainly occurs in liver (5), mRNA encoding FAS, a lipogenic enzyme involved in the synthesis of long-chain fatty acids, is significantly expressed in white adipose tissue as shown in Figure 3. The increases in both LPL and FAS with the same time course between D15 and D30 strongly suggest the differentiation of preadipocytes into mature adipocytes. Such increase is also congruent with chick thermal emancipation that occurs around D15 and allows both parents to feed actively their offsprings and bring them sufficient food energy for fat storage.

As adipocytes acquire the machinery that is necessary for lipid transport and synthesis, they can also synthesize specific proteins such as leptin, resistin and adiponectin. The crucial role of adiponectin which exerts pleiotropic insulin-sensitizing effects prompted us to investigate the occurrence of its mRNA during adipogenesis. Our results show that adiponectin mRNA was detected very early after hatching and the relative abundance of the transcript gradually increased up to 30 days in parallel with adipocyte differentiation (Figure 4). On account of the potential role of this fat-derived peptide in carbohydrate and lipid metabolism of avian species (24), such gradual rise in adiponectin expression may possibly contribute to the maintenance of a high blood glucose concentration during the first month of life (Table 3). Further, adiponectin was also suggested to act

as an autocrine factor in adipose tissues by promoting cell proliferation and differentiation from preadipocytes into adipocytes, augmenting programmed gene expression responsible for adipogenesis, and increasing lipid content and insulin responsiveness of the glucose transport system in adipocytes (13). More experiments are required to clarify the physiological role of adiponectin in penguins.

Proposed scheme for the adipogenic process in Adelie Chick

We have tentatively summarized the major identified events that may chronologically underlie the morphological transformation of adipocytes precursor cells into mature adipocytes as they appear on histological sections of subcutaneous adipose tissue of Adelie chicks (Figure 5). At hatching time, adipose tissue was a loose connective tissue in which it is rather difficult to identify preadipocytes. It is suggested that GH and T_3 play early activating roles at that stage to favour early proliferative steps of preadipocyte precursors. The peak expression of GATA3, a marker of preadipocytes, at D7 implies that it can be a key event at that stage of adipocyte development possibly in relation with the expression of C/EBPα. This timely controlled upregulation of GATA3 strongly suggests an active phase of preadipocyte formation during the first week posthatching. Adipocyte-like cells were visually present from D7 with other cell types including probably fibroblasts, macrophages and endothelial cells but major differentiation and growth of preadipocytes may occur at D15 in connection with several factors such as IGF-1, PPARγ and C/EBPβ gradually leading to functional maturation of metabolic features of adipocytes including lipid storage (LPL, FAS) and late endocrine functions by D30. Accordingly, the size of adipocytes was markedly enlarged until D30 where they appeared as large white empty-looking cells.

In conclusion, the present study has described for the first time the molecular events that may drive the rapid ontogenesis of adipose tissue during the first month posthatching in Adelie penguin chicks. A sequential scheme of gene activation that is slightly different from *in vitro*

studies is proposed. Future studies will clearly have to reconcile animal physiology and cell culture information.

Perspectives and significance.

Given the brevity of the summer breeding season in Antarctica, Adelie chick growth becomes a true race against the clock. Any parameter, such as climatic conditions and food availability, that affects rapid chick growth and building up of energy reserves, is detrimental to juvenile survival during that early period and later at departure to sea, when a massive thermogenic effort is superimposed on moulting energetic cost and fasting. The first weeks post-hatching, when adipose tissue development mainly occurs, are therefore of critical importance for Adelie penguin survival. It is noteworthy that chick mortality is mainly observed during that early period in tight link with changes in environment and food supply. Although global warming has generated ecological changes that increase the accessibility to rich food waters leading to increased Adelie populations (42), a slight perturbation of Antarctic ecosystem may punctually cause dramatic damages in seabird populations. Individual capacities to both develop important fat pads and later use these reserves represent metabolic adaptations that could contribute to select birds able to overcome the energetic challenges of antarctic life. The physiological factors that control the molecular events triggering early fat development deserve to be more fully investigated and in particular those that enable energy sparing when the necessity to conserve and / or store energy as fat is of overriding importance. In this context, the example of barnacle geese that become hypothermic just before their autumn migration in relation with intense fat deposition (8) is of primary interest. There is therefore much to be learned about the relationship between metabolic rate, body temperature, fat deposition and adipose tissue development in birds, particularly in those species such as penguins, that naturally undergo large developmental and seasonal changes in their fat stores.

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FIGURE LEGENDS

Figure 1: Climatic conditions, changes in body mass and morphological data during the first month of life in Adelie penguins. A: Body mass changes of Adelie chicks during their first month of life (mean ± SEM of 8 birds at each age) and ambient temperature recorded at the Dumont d'Urville station. B: Photographs illustrating environmental and behavioural conditions of living at critical steps of the development of Adelie penguin chicks. C: Mass changes of different tissues during the first month of life. D: Correlation between subcutaneous and retroperitoneal white adipose tissue (WAT) mass during the development of Adelie penguin chicks.

Figure 2: RT-PCR analysis of the expression of transcription factors involved in adipogenesis of the Adelie penguin chicks at D1 (hatching), D7, D15 and D30: GATA3 (GATA binding protein 3), C/EBPα and β (CCAAT/enhancer-binding protein α and β), PPARγ (proliferator-activated receptor γ) mRNA abundance relative to β-actin mRNA. Bar values correspond to the mean ± SD values from 8 Adelie chicks in each group (D1, D7, D15, D30). Bars with different letters are significantly different at $p < 0.05$. Representative agarose gels of PCR products obtained from 3 out of the 8 different chicks of each age (D1, D7, D15, D30) are shown. GATA3, CEBPβ, CEBPα, PPARγ were detected at 682 bp, 331 bp, 191 bp and 401 bp, respectively. β actin was detected at 288 bp.

Figure 3: RT-PCR analysis of growth factors stimulating adipogenesis either directly (IGF-1) or *via* the expression of growth factor receptors [IGF-1R, GHR (Growth hormone receptor), TRα (total 3, 5, 3'- triiodo-L-thyronine)] at D1 (hatching), D7, D15 and D30. The relative expression level of each gene was expressed as a ratio to β-actin mRNA levels. Bar values correspond to the mean ± SD values from 8 Adelie chicks in each group (D1, D7, D15, D30). Bars with different letters are significantly different at $p < 0.05$. Representative agarose gels of PCR products obtained from 3 out of the 8 different chicks of each age (D1, D7, D15, D30) are shown. IGF-1R, IGF1,

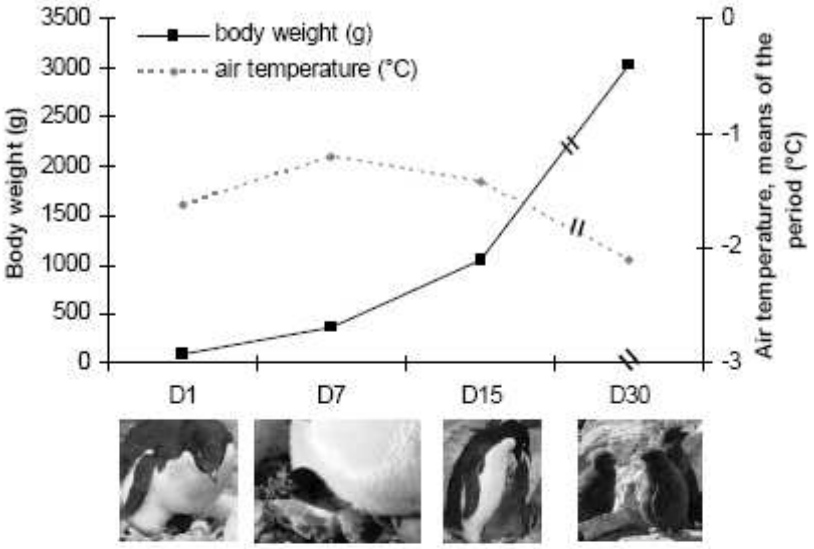
GHR, $T_3R\beta$ were respectively detected at 404 bp, 202 bp, 288 bp and 1082 bp respectively. β actin was detected at 288 bp.

Figure 4: RT-PCR analysis of specific markers of adipocyte differentiation: fatty acid synthase (FAS), lipoprotein lipase (LPL), fatty-acid binding protein (FABP) and adiponectin. The relative expression level of each gene was expressed as a ratio to β-actin mRNA levels. Bar values correspond to the mean ± SD values from 8 Adelie chicks in each group (D1, D7, D15, D30). Bars with different letters are significantly different at $p < 0.05$. Representative agarose gels of PCR products obtained from 3 out of the 8 different chicks of each age (D1, D7, D15, D30) are shown. FAS, LPL, FABP, adiponectin were detected at 979 bp, 584 bp, 109 bp and 735 bp, respectively. β actin was detected at 288 bp.

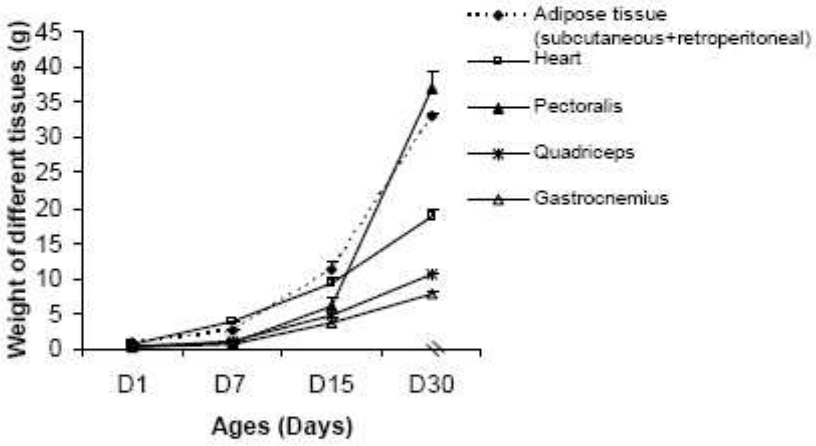
Figure 5: Putative scheme for adipogenesis from hatching to the first month of life in Adelie penguin chicks. Histological evaluation of the conversion of the pre-adipocytes looking cells into mature adipocytes. (Magnification x 400). The main identified events of pre-adipocyte differentiation are presented chronologically. The period of maximal expression of the different genes during the differentiation program is represented by areas labelled by their name.

Figure 1

A


body weight (g)
air temperature (°C)
Body weight (g)
Air temperature, means of the period (°C)
3500
3000
2500
2000
1500
1000
500
0
0
-1
-2
-3
D1
D7
D15
D30

B


Adipose tissue (subcutaneous+retroperitoneal)
Heart
Pectoralis
Quadriceps
Gastrocnemius
Weight of different tissues (g)
45
40
35
30
25
20
15
10
5
0
D1
D7
D15
D30
Ages (Days)

C

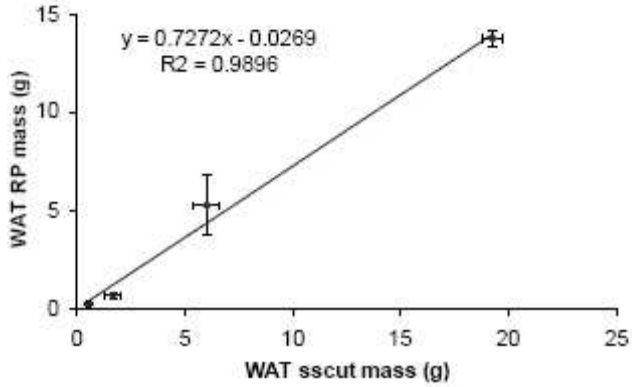

y = 0.7272x - 0.0269
R2 = 0.9896
WAT RP mass (g)
15
10
5
0
0
5
10
15
20
25
WAT sscut mass (g)

Figure 2

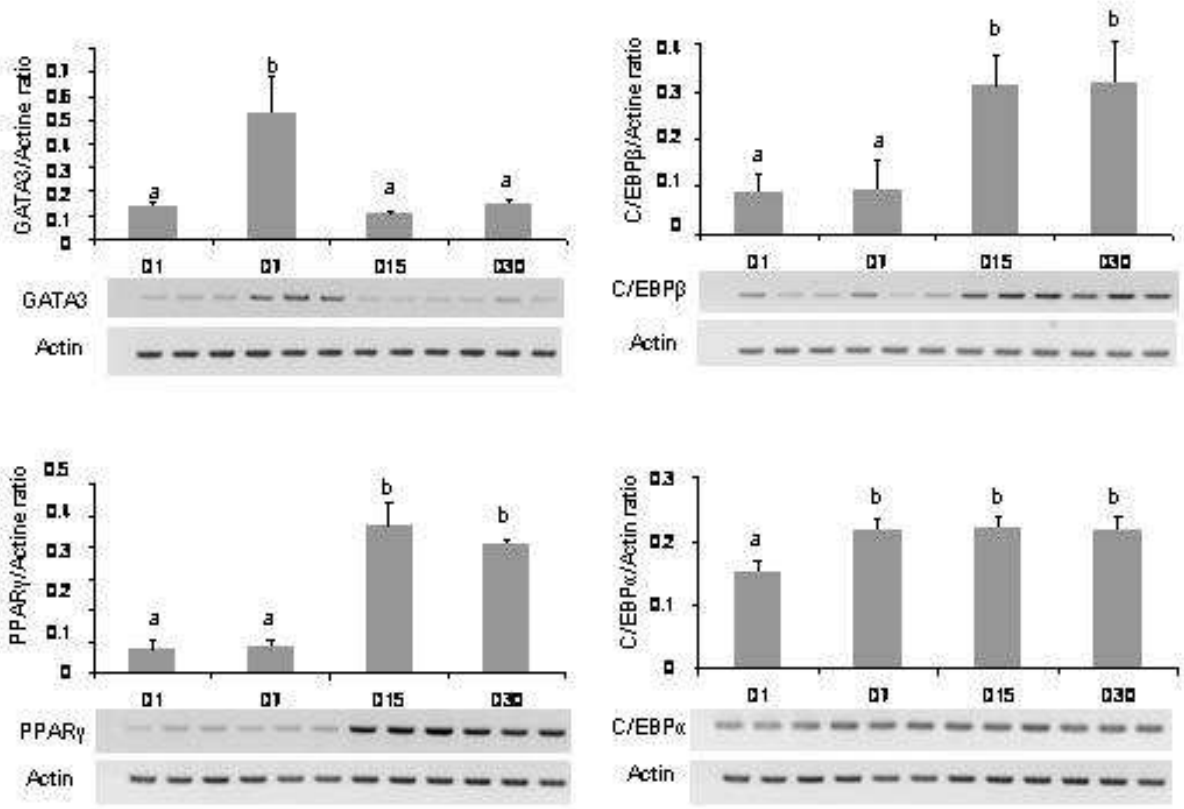

GATA3/Actine ratio
a
b
a
a
D1
D7
D15
D30
GATA3
Actin
C/EBPβ/Actine ratio
a
a
b
b
C/EBPβ
Actin
PPARγ/Actine ratio
a
a
b
b
PPARγ
Actin
C/EBPα/Actin ratio
a
b
b
b
C/EBPα
Actin

Figure 3

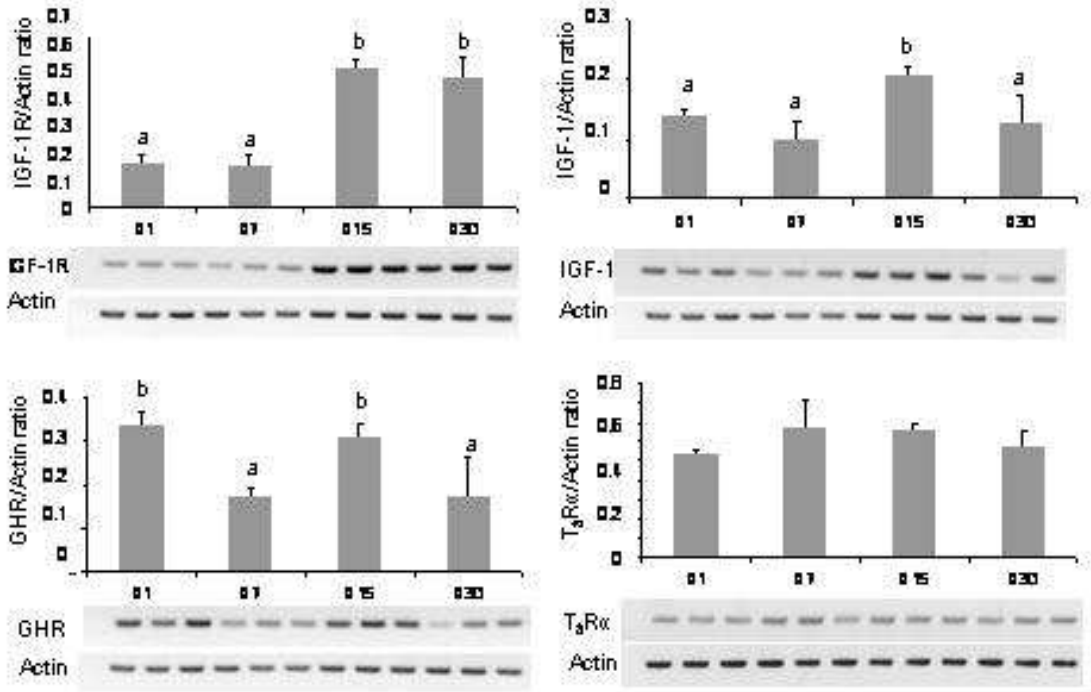

IGF-1R/Actin ratio
IGF-1R
Actin
IGF-1/Actin ratio
IGF-1
Actin
GHR/Actin ratio
GHR
Actin
TaRα/Actin ratio
TaRα
Actin

Figure 4

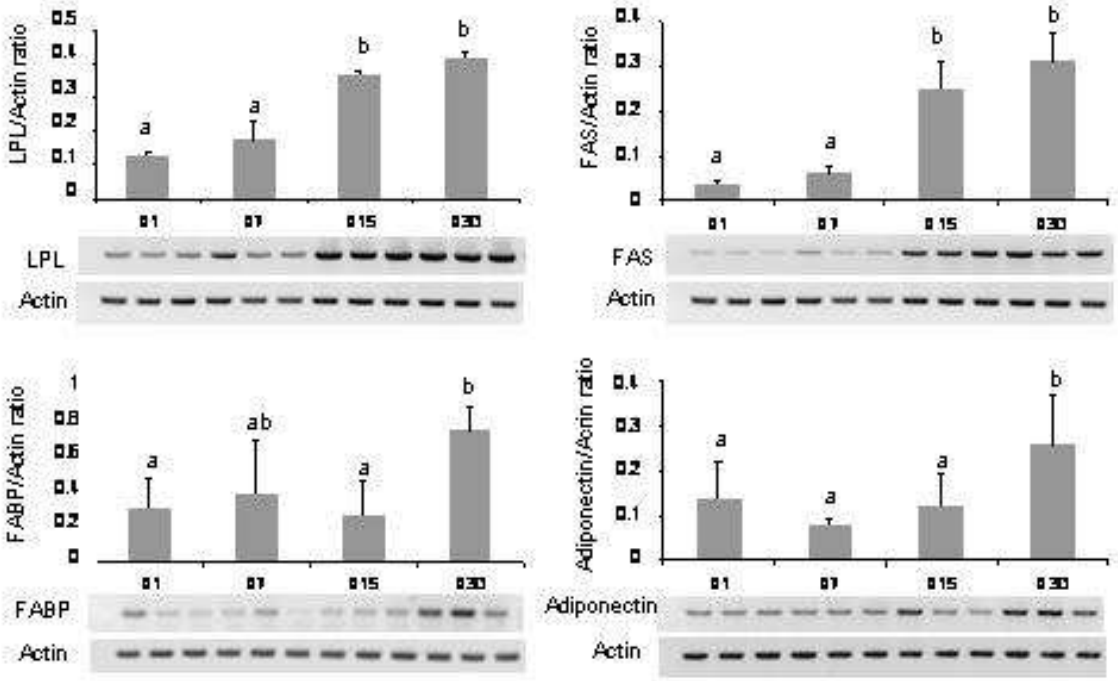
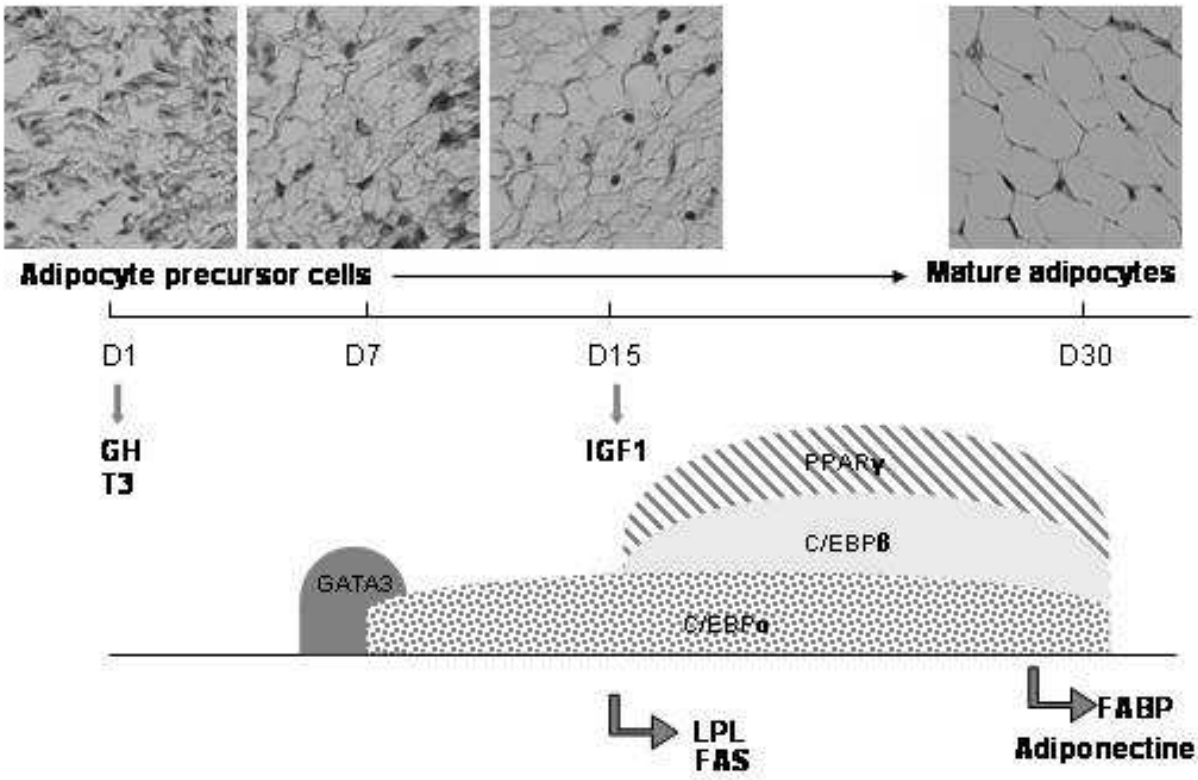

LPL/Actin ratio
0.5
0.4
0.3
0.2
0.1
0
a
a
b
b
D1
D7
D15
D30
LPL
Actin
FAS/Actin ratio
0.4
0.3
0.2
0.1
0
a
a
b
b
D1
D7
D15
D30
FAS
Actin
FABP/Actin ratio
1
0.8
0.6
0.4
0.2
0
a
ab
a
b
D1
D7
D15
D30
FABP
Actin
Adiponectin/Actin ratio
0.4
0.3
0.2
0.1
0
a
a
a
b
D1
D7
D15
D30
Adiponectin
Actin

Figure 5


Adipocyte precursor cells
Mature adipocytes
D1
D7
D15
D30
GH
T3
IGF1
PPARγ
C/EBPβ
GATA3
C/EBPα
LPL
FAS
FABP
Adiponectine