Brown to White Fat Transition Overlap With Skeletal Muscle During Development of Larger Mammals: Is it a Coincidence?

Sunil Pani, Suchanda Dey, Benudhara Pati, Unmod Senapati, and Naresh C. Bal

1School of Biotechnology, KIIT University, Bhubaneswar, Odisha 751024, India
2Center of Biotechnology, Siksha O Anusandhan, Bhubaneswar, Odisha 751030, India

Correspondence: Naresh C. Bal, PhD, School of Biotechnology, KIIT University, Bhubaneswar, Odisha 751024, India. Email: naresh.bal@kiitbiotech.ac.in.

Abstract

In mammals, adipose tissues and skeletal muscles (SkMs) play a major role in the regulation of energy homeostasis. Recent studies point to a possibility of dynamic interplay between these 2 sites during development that has pathophysiological implications. Among adipose depots, brown adipose tissue (BAT) is the major energy-utilizing organ with several metabolic features that resemble SkM. Both organs are highly vascularized, innervated, and rich in mitochondria and participate in defining the whole-body metabolic rate. Interestingly, in large mammals BAT depots undergo a striking reduction and concomitant expansion of white adipose tissue (WAT) during postnatal development that shares temporal and molecular overlap with SkM maturation. The correlation between BAT to WAT transition and muscle development is not quite apparent in rodents, the predominantly used animal model. Therefore, the major aim of this article is to highlight this process in mammals with larger body size. The developmental interplay between muscle and BAT is closely intertwined with sexual dimorphism that is greatly influenced by hormones. Recent studies have pointed out that sympathetic inputs also determine the relative recruitment of either of the sites; however, the role of gender in this process has not been studied. Intriguingly, higher BAT content during early postnatal and pubertal periods positively correlates with attainment of good musculature, a key determinant of good health. Further insight into this topic will help in detailing the developmental overlap between the 2 seemingly unrelated tissues (BAT and SkM) and design strategies to target these sites to counter metabolic syndromes.

Key Words: adipose tissue, brown fat, larger mammals, myogenesis, perinatal development, skeletal muscle

Abbreviations: AT, adipose tissue; BAT, brown adipose tissue; FDG, [18F]-2-fluoro-D-2-deoxy-D-glucose; GH, growth hormone; GHR, growth hormone receptor; IGF, insulin-like growth factor; MRF, myogenic regulatory factor; Mstn, myostatin; Myf, myogenic factor; MYH, myosin heavy chain; MyoD, myogenic differentiation; PET, positron emission tomography; PGC, peroxisome proliferator-activated receptor gamma coactivator; PRDM16, PR domain containing 16; SERCA, sarcoendoplasmic reticulum calcium transport ATPase; SkM, skeletal muscle; SNS, sympathetic nervous system; SR, sarcoendoplasmic reticulum; UCP, uncoupling protein; WAT, white adipose tissue.

Brown adipose tissue (BAT) and skeletal muscle (SkM) are emerging as sites that determine the whole-body metabolic rate in mammals including humans [1–7]. It is proposed that these sites can be targeted to cause increased futile energy expenditure to counter obesity and related comorbidities including type 2 diabetes [8, 9]. SkM constitutes more than 40% of the body mass in healthy individuals, contributing about 50% of daily energy consumption [2, 10, 11]. On the other hand, BAT, although small, can be swiftly activated during physiological stresses like cold to produce heat and contribute significantly to the metabolic status [8]. Hence, both the sites have been implicated as “metabolic sinks” to utilize substrates (sugar and free fatty acid) creating energy demand [12–14]. Interestingly, the transcriptional regulation of BAT during early stages of development overlap with that of the SkM, indicating the possibility of coordinated maturation of these sites [15, 16]. BAT is hallmarked by the presence of large amounts of mitochondria with abundant expression of uncoupling protein (UCP)-1. The developmental pattern of BAT and expression profile of markers (UCP-1 and other transcription factors) are strikingly different between rodents and larger mammals [17, 18]. While rodents rely on active BAT throughout their lifetime, the majority of larger mammals express UCP-1 only during pre/postnatal life and it becomes negligible during adulthood.

Several studies during the last decade have shown the presence of functional BAT in human adults that has catalyzed research in this field. However, subsequent studies found that the gene and protein expression profile of adult human BAT more resembles beige/brite fat of rodents than classical BAT [19]. Based on recent investigations, there is currently an evolving consensus that mouse BAT cannot fully mimic human BAT characteristics. Moreover, the relative abundance of various fat depots exhibits remarkable differences between rodent and nonrodent mammals [20–22]. The ongoing confusion relating to the functionality of BAT and beiging during adulthood can only be resolved by performing studies in animals developmentally closer to humans [23, 24]. In several mammals (ovine and bovine species) this disappearance of BAT is coincident with larger body size. The developmental interplay between muscle and BAT is closely intertwined with sexual dimorphism that is greatly influenced by hormones. Recent studies have pointed out that sympathetic inputs also determine the relative recruitment of either of the sites; however, the role of gender in this process has not been studied. Intriguingly, higher BAT content during early postnatal and pubertal periods positively correlates with attainment of good musculature, a key determinant of good health. Further insight into this topic will help in detailing the developmental overlap between the 2 seemingly unrelated tissues (BAT and SkM) and design strategies to target these sites to counter metabolic syndromes.
Further, this BAT to WAT transition is usually accompanied by the development of SkM in larger mammals including humans. Moreover, significant differences in SkM development have been reported between rodents and larger mammals. The overlap between muscle and BAT development in larger mammals (Fig. 1) has not been highlighted as this phenomenon is not apparent in rodents, which is the animal model predominantly used for research. Therefore, the focus of the present review is to assess structural and molecular aspects of adipose tissue (AT) and SkM development from embryo to adulthood in larger mammals such as ovine, bovine, and porcine animals. We have also gained insight from recent imaging and biochemical studies on humans to deduce the developmental interplay between the sites.

Developmental and Distributional Homology of Adipose Tissue Between Larger Mammals and Humans

In different mammalian species ATs exhibit remarkable variation in their metabolic activity and quantity, ranging between 9% and 50% of total body weight [29, 30]. AT depots contain several types of cells including adipocytes, preadipocytes, fibroblasts, and vascular cells [31]. AT locations have classically been categorized into 2 types: WAT serves primarily as a storage site, and BAT chiefly as a thermogenic site. In addition to visible differences in color and texture, WAT and BAT also display divergences in their function, cellular architecture, and protein expression profiles. While adipocytes in WAT contain a large unilocular lipid droplet of triglycerides for storage during energy surplus states, adipocytes in BAT have multilocular smaller lipid droplets and are rich in mitochondria, which contain the unique protein named UCP-1. The function of UCP-1 is to dissipate the mitochondrial proton gradient and generate heat for maintaining the core body temperature [7].

WAT is mesodermal in origin and shows varied distribution among different mammalian groups [32]. In humans, the major WAT depots are abdominal (omental, perirenal, and intestinal) and subcutaneous (the buttocks, thighs, and abdomen) [12]. Interestingly, the adipocyte content in these WAT depots reaches maximum by roughly 6 months after birth and WAT increases its size mostly by accumulation of fat depending on nutritional availability. Hence, WAT function is highly synchronized with ambient energy status [33]. The volume of the white adipocyte grows with the increasing body size of the organism; however, distribution and the fat to body mass ratio vary across taxonomic groups. While most mammalian species have WAT in a range of 10% to 20% of total body mass, a few species show more extreme WAT content (Table 1). Chimpanzees, for instance, share a body mass range similar to humans but have comparatively much lesser volume of body fat, especially males (0.003-0.005% of total body mass) [43]. Strangely, larger mammals like elephants (mostly used as a tag of obesity) have a comparatively lesser fat to body ratio than humans and rodents [47]. Adipocyte number per body mass is more or less similar during neonatal stages in almost all mammals irrespective of their body size. The size of the adipocytes grows with increasing age and varies across species, as per their food habits and intrinsic metabolic rate. In addition, it is observed for most species, especially humans, that fat to body mass is highest during late adulthood and reduced at older ages. Interestingly, in most species females
have a higher fat percentage than males, which suggests the existence of gender-specific differences in WAT function (described in “Role of Gender in Perinatal Development of SkM and AT”).

In contrast, BAT primarily generates heat by nonshivering thermogenesis in rodents [48, 49] and neonates of larger mammals [50, 51]. BAT is primarily located in interscapular, perirenal, axillary, periadrenal, gonadal, and cervical regions during the fetal and neonatal stages of both larger mammals [19, 41, 52] and rodents [7, 53]. In BAT depots of larger mammals, the abundance of brown adipocytes diminishes while that of white adipocytes increases after a few days of birth [54–57]. In adult humans, the amount of BAT observed is quite insignificant, except for those continuously exposed to cold and in patients with pheochromocytoma with abnormally high adrenergic levels [58–60]. Primary hyperaldosteronism, a condition where the adrenal secretion of aldosterone becomes abnormally high, stimulates adipogenic differentiation and inflammation in perirenal AT depot. Hyperaldosteronism has also been suggested to induce differentiation of brown preadipocytes, causing a major shift in metabolic phenotype [61, 62]. However, studies have shown the presence of metabolically active BAT in adult humans in paravertebral, cervical, axillary, and supravacular regions using [18F]-2-fluoro-D-2-deoxy-D-glucose (FDG) positron emission tomography (PET) scanning [6, 63, 64]. While interscapular BAT is the predominant depot in adult rodents, it is minimal or even absent in the majority of mammalian orders (both precocial and altricial) during adulthood [53, 65]. Studies associating BAT amount in terms of body mass in different mammalian groups have not been performed so far, but phylogenetic analysis of the same has been reported. Here, we performed phylogenetic analysis of UCP-1, believed to be the basis of BAT function, for animals with varying body size from 10 different orders. In 8 orders of mammalian class with both larger and smaller body size, UCP-1 had been inactivated during evolution. UCP-1 evolution was found to be independent of body size, which suggests BAT metabolism and body mass are not related (Fig. 2). However, quantitative studies such as comparative protein expression and FDG-PET scanning analysis will provide more insight on this topic. Recent studies point out that classical BAT is present only in the interscapular region in rodents and human infants, while other BAT depots show adipocyte heterogeneity influenced by the ratio between energy intake and expenditure and have crucial metabolic implications [6, 21, 66].

### Development of Skeletal Muscle

SkMs also undergo several structural and physiological changes during development from fetus to adulthood, largely to fulfill locomotory requirements and to withstand gravity. Muscles dedicated to posture maintenance need sustained contraction (muscle tone) contain mainly slow-twitch fibers (type I), whereas the locomotory muscles meant to support rapid contraction are enriched with fast-twitch fibers (type II) [67]. The fiber type in muscle varies greatly across species depending on their ecological adaptations and shows uniqueness in the developmental pattern.

### Myogenesis and Its Molecular Regulation

SkMs are mesodermal in origin from multipotent mesenchymal stem cells and show different levels of prenatal development depending on the level of precociality [68, 69]. The differentiation of SkM is referred to as myogenesis and occurs at the level of myofibers [70]. In larger mammals, muscle fiber development during prenatal stages is categorized as primary and secondary myogenesis that occurs during early to midgestation, and has been eloquently described by earlier manuscripts [25, 68, 71]. The process of development and differentiation of SkM varies significantly between rodents and larger mammals. In rodents SkM differentiation is activated after birth, whereas in larger mammals it starts during the late gestation period [25]. A common observation during perinatal development is an increase in oxidative capacity of the SkM. Factors that demand such a change are the transition from prenatal to postnatal life, including detachment of the placental supply of oxygen and nutrients, the onset of breathing in the neonate, and the oxygen-carrying capacity of blood [67]. The postnatal growth of SkM only encompasses an increase in fiber size with no net addition of new muscle fibers [25]. However, new muscle fibers can be generated from satellite cells during postnatal life under special circumstances such as injury repair [72]. Differentiated myoblasts from satellite cells fuse with the existing SkM, thereby replenishing the lost/injured part [73].

Myogenesis is a highly regulated process with sequential activation of several key transcription factors. Paired box gene family proteins along with other regulatory factors including wingless and Int induce commitment in mesenchymal stem cells, initiating myogenic regulatory factor (MRF)-4 expression [25, 74]. MRF-4 acts as a key regulator and induces expression of myogenic factor (Myf)-5 and myoblast determination protein, which drive the transition of myogenic precursor cells to myoblasts [75]. Then the myoblasts undergo proliferation, alignment, and fusion to generate immature muscle fibers, a step guided by the transcription factor myogenin (Fig. 3) [74, 78]. During this stage, a cytokine of the transforming growth factor-β family called myostatin (Mstn) plays an inhibitory role on myogenesis in the developing muscle [81]. While the Mstn mRNA starts to express during early myogenesis, reaching its peak during late gestation, several muscles continue to express Mstn until adulthood. Loss of Mstn and enhanced action of its antagonist, follistatin, have been shown to cause “hypermuscularity” in mammals, illustrating the importance of this pathway in regulating muscle development.

### Table 1. WAT to body mass ratio in different mammalian species

| Mammals       | Body mass | Fat percentage | References |
|---------------|-----------|----------------|------------|
|               | Young ones | Adult          |            |
| Mice          | 20-45 g   | 2-4%           | 10-22%     | [34, 35]   |
| Hamster       | 120-160 g | 1-3%           | 18-32%     | [38, 39]   |
| Ring tailed Lemur | 1.7-3 kg | ___            | 14-26%     | [40]       |
| Goat          | 20-50 kg  | 2-5%           | 14-25%     | [41, 42]   |
| Sheep         | 20-50 kg  | 2-5%           | 14-25%     | [39, 42]   |
| Chimpanzee    | 30-40 kg  | ___            | 0.003-8%   | [43]       |
| Human         | 40-100 kg | 15-20%         | 6-30%      | [44]       |
| Dolphin       | 150-650 kg| ___            | 18-22%     | [42]       |
| Camel         | 210-400 kg| ___            | 8-18%      | [45]       |
| Horse         | 400-900 kg| ___            | 8-20%      | [46]       |
| Rhinoceros    | 1800-2500 kg| ___        | 2-25%      | [27]       |
| Elephant      | 1800-6300 kg| ___       | 5-15%      | [47]       |
Interestingly, during postnatal development it has been observed that SkM upregulates expression of genes associated with an oxidative phenotype \cite{83, 84}. The oxidative fibers in SkM are characterized by higher mitochondrial density and vascularization. This process is driven in part by calcineurin, which also plays a crucial role in the postnatal hypertrophy of both slow- and fast-twitch SkM \cite{85, 86}. Further, the expression pattern of myosin heavy chain (MYH) genes shows a unique alteration during the transition from prenatal to postnatal development. MYH genes such as MYH1, MYH2, and MYH7 are upregulated in oxidative fibers (type I and IIa) with a concomitant downregulation of embryonic MYH genes such as MYH3 and MYH8 \cite{67}.

**Sarcoplasmic Reticulum and Calcium Handling**

The routine function of SkM is regulated by the major organelle called the sarcoplasmic reticulum (SR), the primary site of calcium ($\text{Ca}^{2+}$) storage. $\text{Ca}^{2+}$ handling is important not
only for contraction–relaxation of the muscle but also is essential in regulating its metabolism [87]. The development of SR begins in the midgestational period in larger mammals during myoblast formation and innervations [85]. The neuromuscular contacts in primary myoblasts show expression of both subunits of the acetylcholine receptor “α” and “γ.” While γ subunit expression is only limited to fetal development, the α subunit is observed during both prenatal and postnatal development [88, 89]. After the formation of neuromuscular contacts, SR development starts during primary myogenesis, which is marked by the expression of sarcoendoplasmic reticulum calcium trans- port ATPase (SERCA) 2b, the predominant fetal isoform. At the onset of primary myogenesis, the expression of SERCA1 is also observed, which clearly suggests the existence of SR during initiation of SkM differentiation during fetal development [90]. During the perinatal period, based on SERCA1 expression, it has been suggested that the SR develops a close proximal association with the contractile apparatus. The level of Ca\(^{2+}\) storage inside the SR increases after birth due to upregulation of SERCA activity [91] and SR Ca\(^{2+}\)-buffering capacity by increased expression of calsequestrin [92].

Mitochondrial Alterations During Development
A significant increase in mitochondrial biogenesis and its oxidative capacity is observed in SkM during the fetal to neonatal transition [93]. During the same period, the association (both functional and physical) between SR, mitochondria, and the contractile apparatus becomes more intimate [94]. In porcine species, SkM mitochondria are believed to utilize both carbohydrate and lipid for the generation of ATP during fetal development; however, in sheep a preferential use of carbohydrate over lipid is observed [95–97]. After birth, SkM shows marked upregulation of the enzymes associated with fat oxidation, and the mitochondria switch to free fatty acids as a primary fuel source [93, 98, 99]. A significant upregulation in the uptake of O\(_2\) during perinatal development was also observed by Smolich et al [100]. The factor that drives mitochondrial biogenesis during perinatal development has been a matter of debate. While several groups suggested Peroxisome proliferator-activated receptor-gamma coactivator (PGC)-1α to be the primary regulator of mitochondrial biogenesis during myogenic development [101–103], Davies and colleagues observed no significant changes in the expression of PGC-1α during fetal development and concluded it may not be a major factor in this process [93, 104]. Calcineurin, which regulates postnatal myogenic hypertrophy, might also play a role in postnatal SkM mitochondrial biogenesis [85].

Are BAT and SkM Development Synergistically Intertwined?
Developmental Analogy Between BAT and SkM
The functional difference between BAT and SkM is apparent: BAT is the major center for nonshivering thermogenesis while
SkM is responsible for locomotion. Both organs use energy substrates (fat and glucose) as fuel, and due to the major overlap in their function they bear several functional and developmental similarities [16, 105–109]. These include factors associated with oxidative phosphorylation, mitochondrial abundance, and metabolism [16, 106]. Therefore, BAT and SkM show marked overlapping in their mitochondrial protone, including high expression of pyruvate dehydrogenase kinase, carnitine palmitoyl transferase, complex 1 of electron transport chain, and superoxide dismutase. Recent investigations have unraveled an interesting fact that both BAT and SkM are related developmentally as they are derived from a common mesenchymal progenitor cell expressing Myf5. The brown preadipocyte and myocyte lineages diverge by the action of a transcription factor called “PR domain containing 16 (PRDM16)” regarded as the switch between BAT and SkM differentiation. Progenitor cells lacking PRDM16 undergo differentiation towards myogenic lineage, with significant upregulation in the expression of muscle-specific genes. In contrast, induction of PRDM16 in these progenitor cells commits them to the brown adipocytic lineage [110, 111]. Afterwards, myogenic genes are silenced in the brown preadipocytes by Sirtuin1-mediated deacetylation of MyoD, a major transcriptional regulator of myogenesis. It has been observed that brown preadipocytes but not white preadipocytes show a myogenic transcriptional signature, while both preadipocytes share expression of genes associated with adipocyte development, such as Hoxa7, Tbx15, and Hoxc8. Therefore, BAT is developmentally closer to SkM than WAT [106]. This process is summarized in Fig. 4. A few recent discoveries have ignited further research in the field. First is the unearthing of “brown adipocytes” inside adult SkM and that they are derived from SkM precursor cells [115–118]. In addition, the abundance of these adipocytes in SkM positively correlates with resistance against obesity. Second, PET/computed tomography scanning studies by Gilsanz et al have shown a positive correlation between the amount of BAT during neonatal/pubertal stages and SkM mass/size during adulthood [26]. Third, children with scant BAT show metabolic defects in muscles during adulthood. These studies highlight the fact that the functional intertwining of BAT and SkM has major implications in the pathogenesis of metabolic disorders including obesity and diabetes.

Transitions in BAT and SkM During Neonatal, Pubertal, and Adolescent Life

During neonatal development, BAT and SkM undergo drastic transformation in terms of both structure and molecular expression profile [25, 119]. BAT is abundant in infants of several mammalian species [120], while minimized during postnatal life: ~8 weeks in humans [54, 121], ~3 to 4 weeks in goats [120], and ~2 weeks in cattle [55]. However, studies utilizing recent advances in noninvasive imaging technologies have shown that BAT content in humans is transiently elevated during the pubertal stage in children aged 10-13 years of age [77]. The pubertal increase in BAT was gender independent and was observed in the supraclavicular area in 20% to 75% of individuals studied [122]. Interestingly, pubertal reappearance of BAT is correlated positively with SkM development, while negatively with WAT quantity [26, 123]. However, the mechanism behind its re-emergence and its synergistic regulation with SkM still remains an open question. Some studies suggested that common hormonal regulators, such as gonadal hormones, insulin, thyroid hormone, growth factors, and catecholamines, might play roles in codevelopment of the tissues [124, 125]. A possible role of cytokines has been speculated, including myokines such as irisin, which mainly upregulates brown adiposity, and adipokines such as interleukin-6, fibroblast growth factor-21, and insulin-like growth factor (IGF)-1, which enhance SkM development [126–131]. After adolescence and during adulthood, the amount of BAT in humans is drastically reduced. The loss of function of BAT with aging has been suggested to be due to the transformation of brown adipocytes into white in most of the adipose depots [132]. The detailed mechanism for this occurrence is currently lacking. Intriguingly, in humans SkM also undergoes age-related atrophy and loss of muscle mass after 40 years of age [133]. This may coincide with the brown to white transformation of adipocytes in adults and needs to be investigated to gain more insight about the synergistic development of SkM and BAT.

Hormonal and Neural Regulation of SkM and AT Development

The development of SkM and AT depots from perinatal to adulthood is regulated partly by hormones, prominently thyroid, growth hormones (GHs), and steroids [134]. Recent reports showing dysregulation of AT and SkM during prenatal development as a causative factor of metabolic disorders (such as obesity, type 2 diabetes, and fatty liver) during adult life have renewed interest in this field [135–138]. Thyroid hormone is a principal regulator of SkM and AT development during all stages of life. On one hand, it enhances mitochondrial abundance by increasing PGC-1α expression, while also upregulating glucose transporter-4 expression, enhancing glycolytic capacity and thereby boosting muscle and AT metabolism [139]. Thyroid deficiency in the fetus (pig and sheep) modifies gene expression associated with fetal AT development and promotes growth of white adipocytes over brown adipocytes in the perirenal depot [140, 141]. Induced hypothyroidism in the fetus by thyroidectomy has been shown to increase expression of genetic markers of WAT, such as adiponectin, leptin, and lipoprotein lipase [142]. Thyroid hormone also increases UCP-1 and other mitochondrial proteins in cultured BAT from fetal rats and brown adiposity in large mammals during perinatal development [142–144]. A thyroid response element is located in the upstream sequences of the UCP-1 promoter and mediates the thyroid response even in adult BAT in rodents [142, 145]. In addition, thyroid hormone regulates myogenic transcription factors, such as MyoD1 and myogenin, thus mediating muscle development [146, 147]. During muscle maturation, thyroid hormone plays a critical role by modulating expression of SERCA, myosin, and Na⁺/K⁺ ATPase [148, 149]. The thyroid hormone–induced growth and differentiation of myoblasts is also dependent on the activation of growth hormone receptor (GHR) and IGFs [142].

GHs regulate development of both SKM and AT during perinatal stages via GHRs in coordination with IGFs. While the loss of GHRs and IGFs leads to muscle atrophy, their overexpression causes hypertrophy [150]. Interestingly, IGF-1 mRNA is expressed increasingly from early to late gestation, indicating its role in primary and secondary myogenesis, whereas IGF-2 remains high only during early gestation [151]. IGF-1 induces myogenin and MRF4 thereby activating the Met2c gene leading to myogenesis in the fetus [152–155].
In contrast, the role of GH and IGFs during the perinatal development of AT in large mammals has been elusive. While in vitro studies show GH and IGF-I induce adipocyte differentiation and hypertrophy [156–159], in vivo results have been conflicting [160–165]. Some groups suggest a lack of GH in fetal stages predisposes to obesity in later life, whereas others have shown higher GH levels during fetal development leads to elevated white adipogenesis [163–165]. A consensus view suggests that GH and IGFs have primary functions in AT development during early to midgestation and may have only minor fine-tuning roles during perinatal development [161]. Insulin and corticosteroids (especially cortisol) have also been suggested to have a functional interplay with IGFs and GH during the differentiation of both SkM and AT [166]. In WAT, insulin plays an anabolic role, whereas corticosteroids, especially glucocorticoids, play a catabolic role. Hyperinsulinemia was suggested to promote the growth of WAT without changing BAT differentiation; in contrast, cortisol in excess has been shown to induce a lipolytic effect facilitating triglyceride release to the serum [167–169]. This might suggest corticosteroids have distinct functions in different WAT depots, which remains poorly defined. Interestingly, a class of corticosteroid called “aldosterone” has been shown to regulate AT development in a synergistic manner. Studies suggest that aldosterone induces BAT differentiation from a preadipocyte cell line (T37i) via mineralocorticoid receptors. In return, BAT is proposed to release angiotensinogen, which subsequently augments aldosterone production via the renin–angiotensin–aldosterone pathway. This shows that aldosterone also plays a critical role in adipose metabolism in addition to its better known function in electrolyte homeostasis [170, 171]. Sex steroid hormones are known to regulate development of both AT and SkM during perinatal, pubertal, and adult life. A positive correlation is observed between gonadal hormones and the thermogenic/oxidative capacity of BAT and SkM [124, 172]. BAT expresses estrogen and androgen receptors abundantly, indicating that these steroids regulate BAT activity [173]. Decreased production of sex steroids has been proposed to reduce BAT thermogenesis due to unhindered action of cortisol during adulthood [4, 174, 175].

Figure 4. Schematic representation showing synergistic interplay between muscle and brown fat development. During development, BAT and SkM share a common Myf5+ progenitor cell while WAT originates from Myf5− progenitors. PRDM16 is a key transcriptional switch that induces commitment towards the BAT lineage, while inhibiting the SkM lineage [16, 105]. Thyroid hormone promotes metabolism and programs both sites to carry out adaptive thermogenesis. Thyroid hormone is a synergistic supplement alongside the SNS for enhancing UCP1 expression and structural modifications that are required for fully functional BAT in mice [112]. In an analogous manner, thyroid hormone also orchestrates the long-term adaptation of SkM, including the regulation of several key proteins like SERCA [113]. Moreover, BAT and SkM exhibit comparable regulation of oxidative metabolism through mitochondrial proteins, hormonal action, sympathetic innervations. [114]. All these features reflect that BAT is developmentally and functionally closer to SkM than WAT. Created with biorender.com.
The development of BAT and SkM is also regulated by the sympathetic nervous system (SNS). During the perinatal period, the SNS enhances the BAT phenotype by promoting UCP-1 expression and SkM activity by regulating the expression of proteins involved in fiber typing and intracellular Ca\(^{2+}\) channels (Fig. 4) [149, 176]. The SNS, via catecholamines and by employing other hormones such as thyroid hormone [112, 177] and prolactin, upregulates UCP-1 expression in BAT during late gestation [178]. During late neonatal stages, local adrenergic signaling is reduced partly due to increased intracellular expression of catecholamine-degrading enzymes, such as monoamine oxidase and catechol-O-methyl transferase, contributing to decreased BAT activity [179]. The SNS coordinating center, the hypothalamus, also influences brown adipogenesis via peptides like orexin [180]. Sympathetic activity is also suggested to regulate almost all aspects of SkM function including vascularization, contractility, and metabolism [181, 182]. Increasing evidence shows that sympathetic neurons are part of the neuromuscular junction and influence SkM development and expression of metabolic sensors like PGC-1α [183]. The presence of sympathetic neurites has been reported for SkM such as the diaphragm, extensor digitorum longus, gastrocnemius, and tibialis anterior based on the expression of tyrosine hydroxylase and neuropeptide Y, 2 markers of sympathetic neurons. Expression of tyrosine hydroxylase and neuropeptide Y rise during neonatal SkM development in rodents, indicating an increase in sympathetic input [184]. However, developmental regulation of SkM by the SNS in larger mammals is underexplored.

**Role of Gender in Perinatal Development of SkM and AT**

Sexual dimorphism in adiposity and musculature is common among adult mammals: females having greater adiposity and males exhibiting greater musculature. However, the age at which sexual dimorphism appears and its role in the developmental interplay of SkM and AT in early life is less explored. Longitudinal cohort studies show that gender-based differences in body composition are minimal at birth but acquire multifold differences during the first 6 months of the postnatal period [185]. In this period, female infants increase adiposity compared with males, while male infants increase lean body mass. These studies also demonstrate that puberty is another major window of time during which sexual dimorphism is further pronounced [31, 186, 187]. The association of adiposity accumulation during early infancy with adolescence and adult obesity supports the idea that programming during this critical window of development influences metabolism of AT throughout life [188]. Other studies in rodents have reported that gender-specific differences in SkM and AT arise during prenatal development [189, 190]. Estrogen receptor knockout mice show increased adiposity in both sexes, while androgen receptor knockout exhibits no effect on adiposity but instead impairs muscle development [191–194]. These data suggest that estrogen is an adiposity-promoting hormone while androgen is an enhancer of musculature [195, 196]. In ovine and bovine species, male fetuses exhibit greater myogenesis, whereas female fetuses show greater intestinal development with no difference in adipogenesis or fibrogenesis [197].

Studies have shown that muscles from male fetuses have higher mRNA expression of myogenic markers such as Catenin Beta 1 and Myogenic differentiation (MyoD)1 [190]. Other studies have suggested that the sexual divergence in AT and SkM is initiated during early infancy [198]. Longitudinal cohort studies of healthy term babies indicate higher subcutaneous AT volumes in females during the first 2 to 3 months than in males [187, 198, 199]. These gender-based differences can arise due to sex hormones as estrogen promotes deposition of AT in the gluteofemoral regions, whereas testosterone promotes deposition in abdominal regions [200–203]. Moreover, androgen receptors are expressed more abundantly in intra-abdominal AT than in subcutaneous AT [204]. Interestingly, there is a surge in sex-related hormones immediately after birth, termed “mini-puberty of early infancy” [205]. During the first week after birth, the circulating levels of luteinizing hormone, follicle-stimulating hormone, and sex hormones (testosterone and estrogen) tend to rise. The rise in sex hormones is also accompanied by increased levels of aldosterone and cortisol, resulting in hyperaldosteronism [206]. The levels of these hormones remain high for about 6 months in males and 2 to 3 years in females [205, 207, 208]. This period of mini-puberty coincides with the commencement of sexual dimorphism of SkM and AT in humans. The rise in testosterone has been suggested to be the possible reason behind enhanced musculature in males. The role of mini-puberty in the enhanced musculature in males has also been observed in male monkeys, as muscle mass diminished after blocking neonatal secretion of testosterone and gonadotrophins [209]. Compromised mini-puberty has also been suggested to trigger obesogenic metabolic imprinting [210]. Thus, mini-puberty along with SkM/AT development are possibly correlated with processes that determine the metabolic status during adulthood. Despite this, our understanding of the origins of sex differences in AT and SkM is largely speculative, as most studies to date have focused on rodents. An increased number of studies on human or physiologically closer mammals will define the exact role of gender in development of AT and SkM.

**Conclusion**

In the last 2 decades, there has been significant progress in the understanding of developmental regulation of AT and SkM using rodents as animal models. The discovery of beige adipocytes and active BAT in adult human has further catalyzed research in this field. However, there are several key questions that still remain poorly explored, especially related to larger mammals as these studies are resource intensive and time consuming. Some of the major unanswered questions are (1) the mediators of interplay between BAT and SkM: cytokines, miRNA or hormones; (2) the mechanism behind this interplay: while studies on rodents give a faint idea regarding the synergism between both sites, studies in larger mammals will reveal mechanistic details; and (3) the regulators of the synergism between BAT and SkM: while previously it was believed hormones and the central nervous system to be the primary regulator, recent studies project SNS to be a crucial regulator of SkM activity. This points out a possible role of the SNS behind the synergism between BAT and SkM. Further studies in larger mammals (rather than rodents) are essential to resolve these unanswered questions.

**Acknowledgments**

We thank Bijayashree Sahu, Gourabamani Swalsingh, and Punyadharma Pani for their scientific inputs towards the outline.
of the review, and for their critical comments on the manuscript. We thank Dr. Leslie Rowland for careful editing and grammatical corrections.

**Funding**
This work has been partly funded by Science and Engineering Research Board (SERB), DST, India (grant no. ECR/2016/001247) and DBT, India (grant no. BT/RLF/Re-entry/41/2014 and BT/PR28935/MED/30/2035/2018) to N.C.B. S.P. and S.D. are recipients of Senior Research Fellowships from Indian Council of Medical Research (ICMR) vide grant no 45/3/2019/PHY/BMS and AMR/Fellowship/13/2019/ECD-II respectively.

**Disclosure**
The authors have nothing to disclose.

**Data Availability**
All the data generated has been included in the submitted article.

**References**

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