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Cellularity Description of Adipose Depots in Domesticated Animals

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Abstract

Cellularity of adipose tissue in domesticated animals varies not only with species, sex, age and management conditions but also with depot. Differences in depots are important in animal production because of the economic and welfare implications and in humans in relation to obesity. The final amount of fat and its composition depends on the differentiation of mesenchymal multipotent precursor cells into mature adipocytes (adipogenesis) capable of fatty acid and triglyceride synthesis (lipogenesis), both processes being regulated by different key adipogenic and lipogenic genes, some of are well known and have been described. Histologically, differences can be classified as hyperplasia (an increase in adipocyte number) and hypertrophy (an increase in adipocyte size), processes that can produce adipocyte size distributions that are not necessarily Gaussian. A detailed description of the type of adipocyte size distribution can help distinguish the different adipocyte populations within depots and characterise each not only in terms of the size but also the number of the constituting cells. This description can help better understand the development and role of the different depots. It can also help when analysing causal relationships with adipogenic drivers and lipogenic enzymes involved in lipid metabolism.

Keywords: adipocyte, adipogenesis, lipogenesis, hyperplasia, hypertrophy, size, number, distribution

1. Introduction: cellularity of adipose tissue

Adipose tissue is a specialised connective tissue mesenchymal in origin formed by an association of cells called adipocytes, namely, cells that specialise in accumulating lipids. Adipose

tissue plays a fundamental role in maintaining the energy balance in animals, which entails storing energy in the form of fat (triglycerides) during periods of high energy intake and releasing energy through lipolysis during periods of caloric restriction [1]. However, certain discoveries made more than 20 years ago revealed that it is also a highly active endocrine tissue that secretes important substances, namely, adipokines and adipocytokines (*leptin*, *adiponectin*, *tumour necrosis factor alpha* or *TNF-α*, etc.), enabling it to communicate with different organs and demonstrating its involvement in such biological processes as neuroendocrine function and immune function [2, 3].

There are two main types of adipose tissue, white adipose tissue and brown adipose tissue, formed mainly of white and brown adipocytes, respectively. These tissues are differentiated not just only in terms of their colour but also in terms of their morphology, partitioning, genes and function [4]. The third adipocyte type known as "beige/brite" adipocytes growing in white adipose tissue in response to hormonal stimuli and cold has recently been described in rats and humans [5], as well as in cattle [6] and sheep [7].

Brown adipose tissue consists of small adipocytes (25–40 μm in diameter), which are multilocular, i.e., they contain several lipid droplets surrounded by large numbers of mitochondria; their main functions are thermogenesis, i.e., heat production, and thermal homeostasis [8]. This type of adipose tissue is typical in newborn animals in most species and in adult hibernating mammals [9, 10].

White adipose tissue is the predominant form in adult animals and is the main focus of the considerations set out here. White adipose tissue is composed mainly of adipocytes and a stromal vascular fraction comprising macrophages, capillary endothelial cells, undifferentiated preadipocytes, pluripotent stem cells and fibroblasts. This stroma is responsible for tissue homeostasis [11]. The adipocytes in white adipose tissue are ordinarily large (in cattle they can reach sizes of up to 180 μm in diameter) [12], and they have few mitochondria, their cytoplasm being almost entirely taken up by a single fat vacuole [13, 14]. In addition to its endocrine function, this fatty tissue subtype also plays a structural role, providing mechanical support and protection for certain parts of the body [3, 15, 16].

2. Adipose depots in domesticated animals

Adipose tissue is located in specific deposits, or depots, that are similar in mammals, though the size and composition of the depots vary with species, age, sex, diet, etc. [17]. The main depots are the visceral depots, comprising the kidney knob and channel fat (covering the kidneys and the pelvic cavity), mesenteric fat (surrounding the bowel) and omental fat (surrounding the pre-stomachs), the subcutaneous (SC) depot (covering the outside of the carcass), the intermuscular depot (located in between the muscles) and the intramuscular (IM) depot (infiltrated within the muscles) [18]. For example, Joy et al. [19] observed that omental fat accounted for 10%, kidney knob and channel fat 8.13%, mesenteric fat 8.01% and SC fat 25.3%, with intermuscular fat accounting for 42.6%, in lambs with a live weight of 23 kg fed a concentrate diet. Therefore, the importance of the IM depot is small compared with that of the other depots in quantitative terms.

The relevance of fatty tissue is the effect of the proportions and compositions of the different depots on production efficiency, nutritional value and meat and carcass quality. Fat is present in meat in the form of intermuscular fat, membrane phospholipids and IM and SC fat [20]. From a production standpoint, not all the depots have the same value, with the depositing of IM fat, or marbling, being desirable because of its beneficial effects on sensory attributes and hence its decisive role in determining meat quality [21]. In contrast, the accumulation of excess fat in certain depots has an adverse effect by increasing production costs while decreasing product quality [15, 22]. For instance, visceral fat is mostly discarded at slaughter, while an excess of SC fat lowers the commercial value of carcasses. Subcutaneous fat is also of concern to consumers, because excess SC fat and a high saturated fatty acid (SFA) content are viewed as detrimental to human health [15, 23].

Therefore, the current trend is towards producing lean meats with optimum fatness levels, in particular suitable amounts of IM fat. Additionally, a certain amount of covering, or subcutaneous, fat is necessary to reduce carcass moisture loss and keep the carcass from drying out while also guarding it against potential bacterial contamination [24].

Intramuscular fat composition is especially important because of its impact on meat quality from the standpoint of human health. IM fat is formed mainly by phospholipids, which have a high polyunsaturated fatty acid (PUFA) content, and by triglycerides, consisting mostly of SFAs and monounsaturated fatty acids (MUFAs) [25]. The phospholipid content in the muscle is relatively constant because of phospholipid's role as structural constituents of the cells, so it is not directly related to fatness. What is more, it is not particularly influenced by species, breed, diet or age, though the n−6/n−3 PUFA ratio may experience some variation according to the diet [26]. Unlike phospholipids, triglycerides in IM tissue may vary considerably from 0.2 to 5%, depending on accumulated fat levels, muscle location and breed [27]. Triglycerides make up most of the lipids (around 90%) in SC adipose tissue, and the PUFA content, especially eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), is low because phospholipids make up just a small proportion [28–30].

As already indicated, PUFAs are selectively deposited among the phospholipids associated with muscle cell membrane, which may furnish opportunities for modifying muscle PUFA content in ways beneficial to human health without increasing the amount of IM fat [20, 31].

3. Adipogenesis and lipogenesis

Adipogenesis is the process of differentiation by which multipotent mesenchymal precursor cells turn into mature adipose cells [32, 33]. Mesenchymal stem cells are fibroblast cells mesodermal in the origin capable of acting as precursors not only for adipose tissue but also for muscle, bone, cartilage and tendon [34]. There are two stages in the process of adipocyte differentiation: determination and terminal differentiation (**Figure 1**).

The first stage encompasses the mechanisms, whereby the pluripotent stem cell takes on the characteristics of the adipocyte lineage and involves transformation of the stem cell into a preadipocyte that is morphologically still the same as the precursor. During this transition, the
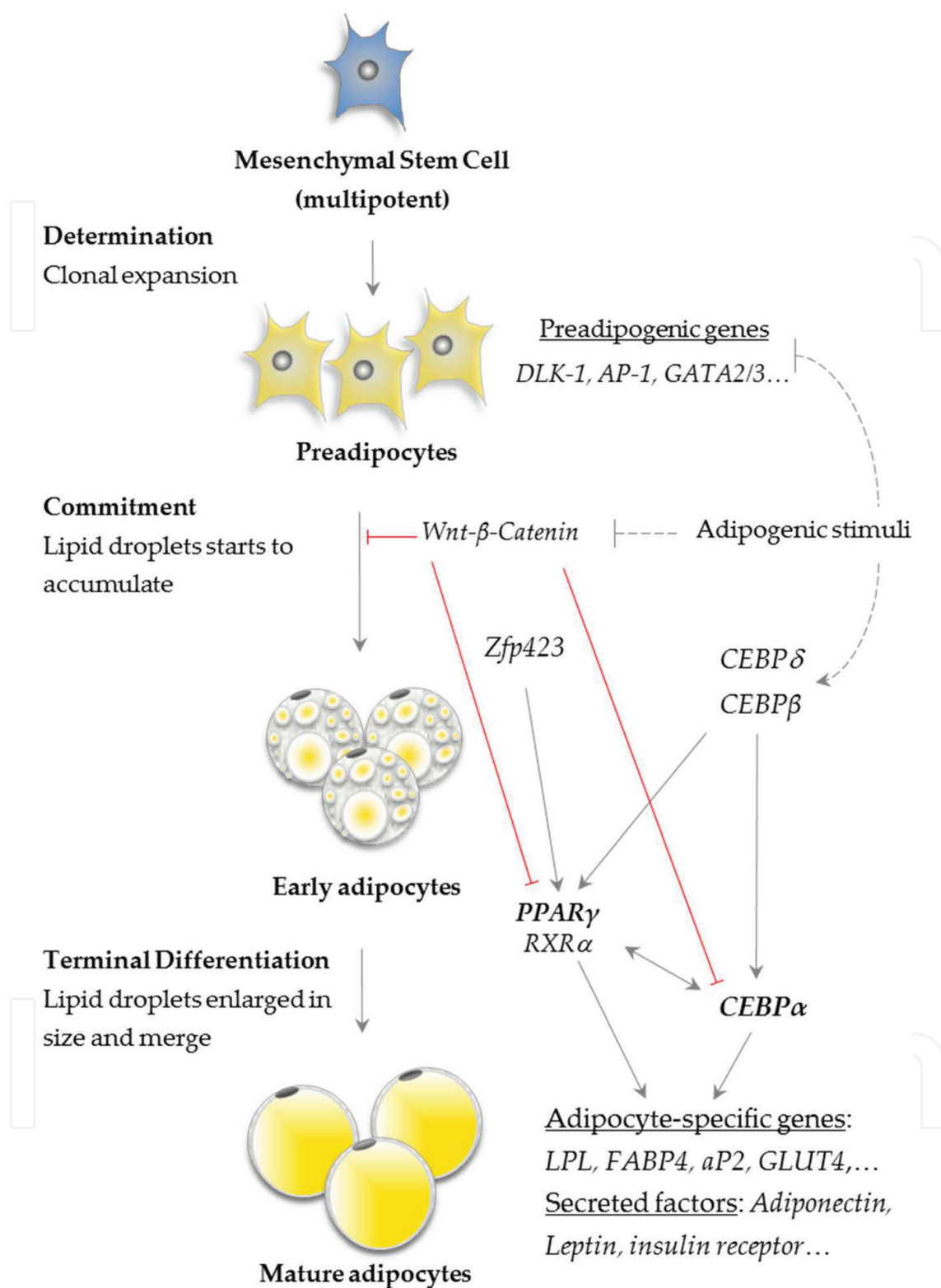
Figure 1. Schematic representation of the transition process from mesenchymal stem cell to mature adipocyte and transcriptional cascade during adipogenesis. *DLK-1 = Delta-like 1 homolog; AP-1 = activator protein-1; GATA2 and 3 = GATA binding proteins 2 and 3; WNT = wingless-type MMTV integration site family members; Zfp423 = zinc finger protein 423; CEBP a, β and δ = CCAAT/enhancer-binding protein a, β and δ; PPARg = peroxisome proliferator-activated receptor gamma; RXRa = retinoic X receptor; LPL = lipoprotein lipase; FABP4 = fatty acid-binding protein; aP2 = adipocyte protein 2 and GLUT4 = glucose transporter type-4.*

cell loses its ability to turn into other types of cells [35]. Both positive (*zinc finger protein 423* or *Zfp423*; *activator protein-1* or *AP-1*) and negative (*delta-like 1 homologue* or *DLK1*, *GATA-binding proteins 2* and *3* and *wingless-type MMTV integration site family members* or *WNTs*) regulatory factors are known to take part in the initial stage, which is still poorly understood [36].

In the second stage of terminal differentiation, the preadipocytes acquire the complement of proteins needed for lipid transport and synthesis, insulin sensitivity and the ability to secrete adipokines and turn into mature adipocytes [9, 37]. This is accompanied by the accumulation of fat inside the cell and a change in morphology into a more globular shape [36]. This second stage is viewed as a cascade of transcriptional events in which the first wave consists of induction of *CCAAT/enhancer-binding protein (CEBP) β* (*CEBPβ*) and *δ* (*CEBPδ*), which activate expression of the central adipogenesis factors *peroxisome proliferator-activated receptor gamma* (*PPARγ*) and *CEBPα*. These transcription factors stimulate expression of genes involved in lipogenesis, such as *lipoprotein lipase* (*LPL*), *fatty acid-binding protein* (*FABP4*) or *adipocyte protein 2* (*aP2*) and *glucose transporter type-4* (*GLUT4*) [38, 39].

A variety of extracellular factors are capable of acting on the regulation of the above-mentioned transcription factors and are able to determine whether preadipocytes start the process of differentiation or remain quiescent. The activating factors include insulin, glucocorticoids, mono- and polyunsaturated fatty acids (which appear to be *PPARγ* activators) and prostaglandins [40].

Lipogenesis comprises the processes of fatty acid synthesis and triglyceride formation and takes place primarily in the liver, the white adipose tissue, and in the case of lactating females, the mammary gland. The biochemical mechanisms occurring in these tissues are similar, but the role of each of these processes varies by animal species. Adipose tissue is the main location of fatty acid synthesis in ruminants, and indeed in sheep, this tissue is responsible for over 90% of fatty acid (FA) biosynthesis [41]. As in ruminants, adipose tissue is the main site of fatty acid synthesis in pigs [42]. By contrast, in poultry it is the liver that is most active in de novo lipogenesis, while in rats, mice and rabbits, activity levels are similar in both tissues.

The fatty acids thus formed may be used to make cell membrane-building phospholipids, they may be used as precursors for biologically active metabolites or they may be used in the synthesis of triglycerides to store metabolic energy. This last-mentioned process is carried out by the endoplasmic reticulum in adipocytes and involves esterification of activated fatty acids (acyl-CoA molecules) through the action of acyl-CoA synthetase (ACS) and glycerol-3-phosphate from glucose catabolism.

Fatty acids from de novo synthesis are, as mentioned above, a source of acyl-CoA, but fatty acids ingested in the diet imported by *LPL* or released by lipolysis may also be used. Glycerol-3-phosphate is obtained by the action of the G3PDH enzyme on dihydroxyacetone phosphate (DHAP), though a small quantity may be produced by the action of glycerol kinase on glycerol [43].

4. Adipocyte hyperplasia and hypertrophy

Adipose tissue plasticity is high, and growth is produced by a combination of two mechanisms: hyperplasia, or an increase in the number of cells, and hypertrophy, or an increase in

cell volume. Hyperplasia is a proliferation of preadipocytes and their subsequent differentiation and occurs primarily in the animal foetus and postnatal period. Hypertrophy also takes place during this period, whereby adipocyte volume increases through the accumulation of lipids in the form of triglycerides, formed by the esterification of glycerol-3-phosphate, which is synthesised from glucose and fatty acids (from the diet or synthesised de novo) [4, 13, 44, 45].

After birth and the postnatal period, adipose tissue growth is mainly by hypertrophy of existing adipocytes and the activity of lipogenic enzymes [46]. Nevertheless, adipose tissue size in animals is not necessarily limited by the hyperplasia taking place at an early age, inasmuch as adipocytes can stimulate adipogenesis when a given percentage of adipocytes have reached their maximum volume, thereby inducing an increase in hyperplasia and/or promoting lipid accumulation by preadipocytes which had been quiescent until then [13, 43, 47].

Both hyperplasia and hypertrophy can be influenced by factors such as sex, breed, age, physical condition, diet type and amount, anatomical location of the adipose depots, etc. [48]. As a whole, fat deposition increases with animal weight and age, though development of the different fat depots is not uniform from either a quantitative or a temporal standpoint [49]. Generally speaking, the kidney knob and channel fat depot are the first to grow, followed by the intermuscular and SC depots and, lastly, the IM depot [13, 15]. In sheep, hyperplasia in the kidney knob and channel fat depot is complete approximately 60 days after birth, and further growth is ascribed to hypertrophy. Both processes contribute to growth of the intermuscular depot, with hyperplasia occurring until around 100 days after conception. In contrast, hyperplasia and hypertrophy together may contribute to SC depot growth until lambs turn 12 months of age [13, 44].

IM fat is a late-developing depot, and fat deposition in the IM depot would appear to depend mainly on hyperplasia [23, 50, 51]. In older animals the rate of fat accumulation outpaces muscle growth, in contrast to the situation at younger ages, and as a result, intramuscular fat deposition necessarily takes place later in life [23].

As already mentioned, adipose depots do not all grow at the same time or at the same rate, and adipocyte size therefore varies from one depot to another [22]. As a general rule, mean adipocyte size by depot in sheep [50, 52, 53] and cattle [54–57] follows this order: omental > kidney knob and channel fat > SC > intermuscular > IM. Furthermore, lipogenic activity in the different fat depots also varies, and this would appear to be related to adipocyte size and thus for the most part follows a similar order [22].

Intramuscular adipose tissue makes up a single depot, because it can be distinguished from other fat depots by its location within the perimysial connective tissue next to the myofibrils, and in addition its metabolism displays certain differences as compared to the other depots. Rates of fatty acid synthesis by intramuscular adipocytes are some 5–10% of the rate observed in the SC depot, which means that the fatty acid synthesising ability of the IM depot is low, as is its ability to break down fatty acids [58]. Gondret et al. [59] carried out proteomic analysis and reported that various lipogenesis, lipolysis, fatty acid oxidation and basal metabolic rate indicators were lower in IM tissue than in SC tissue.

Furthermore, intramuscular adipocytes chiefly use glucose and/or lactate as a carbon source for lipogenesis, whereas SC adipocytes use acetate [60]. Consequently, as Hausman et al. noted [58],

there is evidence that the IM and SC tissues are metabolically distinct, with these differences manifesting at the cellular level (preadipocytes and adipocytes), such that, for instance, the proliferation and differentiation potency of bovine intramuscular preadipocytes is lower than that of bovine subcutaneous preadipocytes [61, 62].
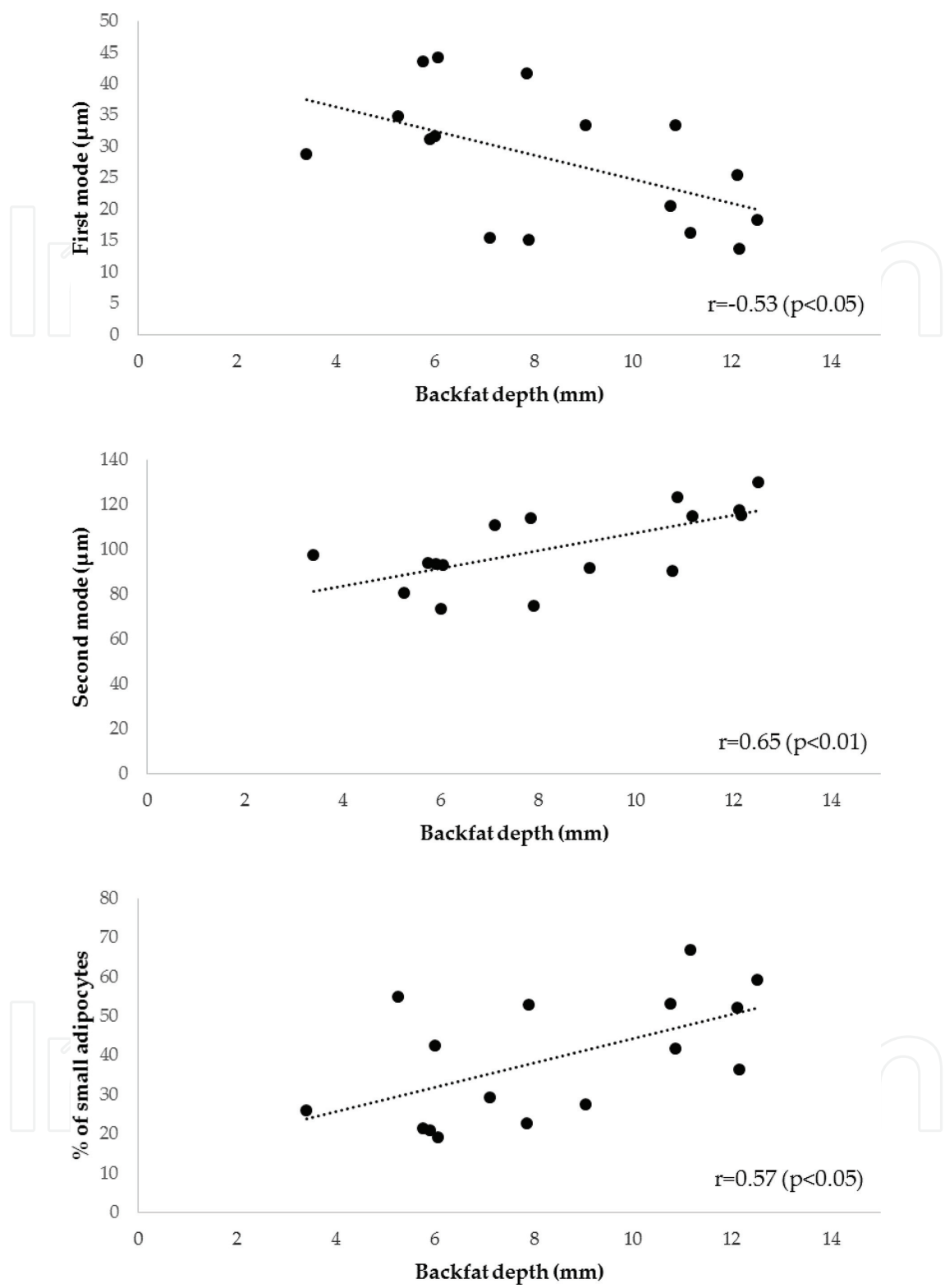
5. Adipocyte size distribution

Adipocyte size in domesticated animal populations has been estimated for decades [63]. At the present time, there are different methods of evaluating adipocyte size, by means of electronic measurement following fixation with osmium tetroxide, by means of histological analysis and by means of collagenase digestion. These last two methods are constrained as to the numbers of adipocytes that can be analysed per sample (around 250), but they are less expensive and require less expenditure on equipment [64]. The collagenase digestion method, in which collagenase is used to dissolve the connective tissue surrounding the adipocytes [65], is frequently used in animal studies [48, 66–69]. Succinctly, samples taken from animals at slaughter are stored in Tyrode's solution at 39°C, blood vessels and connective tissue are removed and the samples are washed in saline solution and digested using collagenase under incubation at 39°C for 1 and a half hours. The solution is then filtered through an 850 μm filter, microscope slides are prepared and the diameters of a sample of at least 200 of the adipocytes thus obtained are measured using image analysis.

Irrespective of the method employed, for many years now, the size distributions of adipocytes in different animal species have sometimes been observed to fit a normal distribution [48, 53, 70], but this is not always the case. Bimodal distributions have been described [55, 71–73], and this may, though not always [74], indicate the presence of a heterogeneous cell population [75]. The bimodal distribution of adipocyte size could thus be an indication that both hyperplasia and hypertrophy are taking place at the same time or that factors contributing to heterogeneity during adipocyte hypertrophy are at work. Growth in adipocyte size may increase local hypoxia, resulting in cell death as well as, after a certain critical volume has been reached, in the secretion of new adipocyte recruitment factors [76]. Another possible source of the bimodal distributions could be that the metabolic activity of adipocytes depends on individual cell surface size, since triglyceride exchange with the extracellular milieu occurs across the surface, and consequently differences in lipolytic and lipogenic fluxes could give rise to adipocyte populations that differ in size without hyperplasia necessarily taking place [77].

In any case, proper description of adipocyte size distributions is needed to be able to identify the factors underlying the differences in adipose tissue growth between groups of animals. If a distribution is bimodal, describing adipocyte size on the basis of the mean and the standard error of the mean is not meaningful, and using the mean to compare differences in experimental treatments could lead to erroneous conclusions. Compared with simply calculating mean cell size and the total number of adipocytes, studies that deal with adipocyte distributions are better at explaining the mechanisms involved in fat development [78]. For this reason, various approaches to evaluating adipocyte size distributions have been put forward, e.g., lognormal distribution fitting [72], normal distribution mixtures [79] and combinations of exponential

Figure 2. Relationship between the first mode, second mode and percentage small adipocytes and backfat thickness in the outer subcutaneous layer in brood sows (data after Abadía et al. [67]).
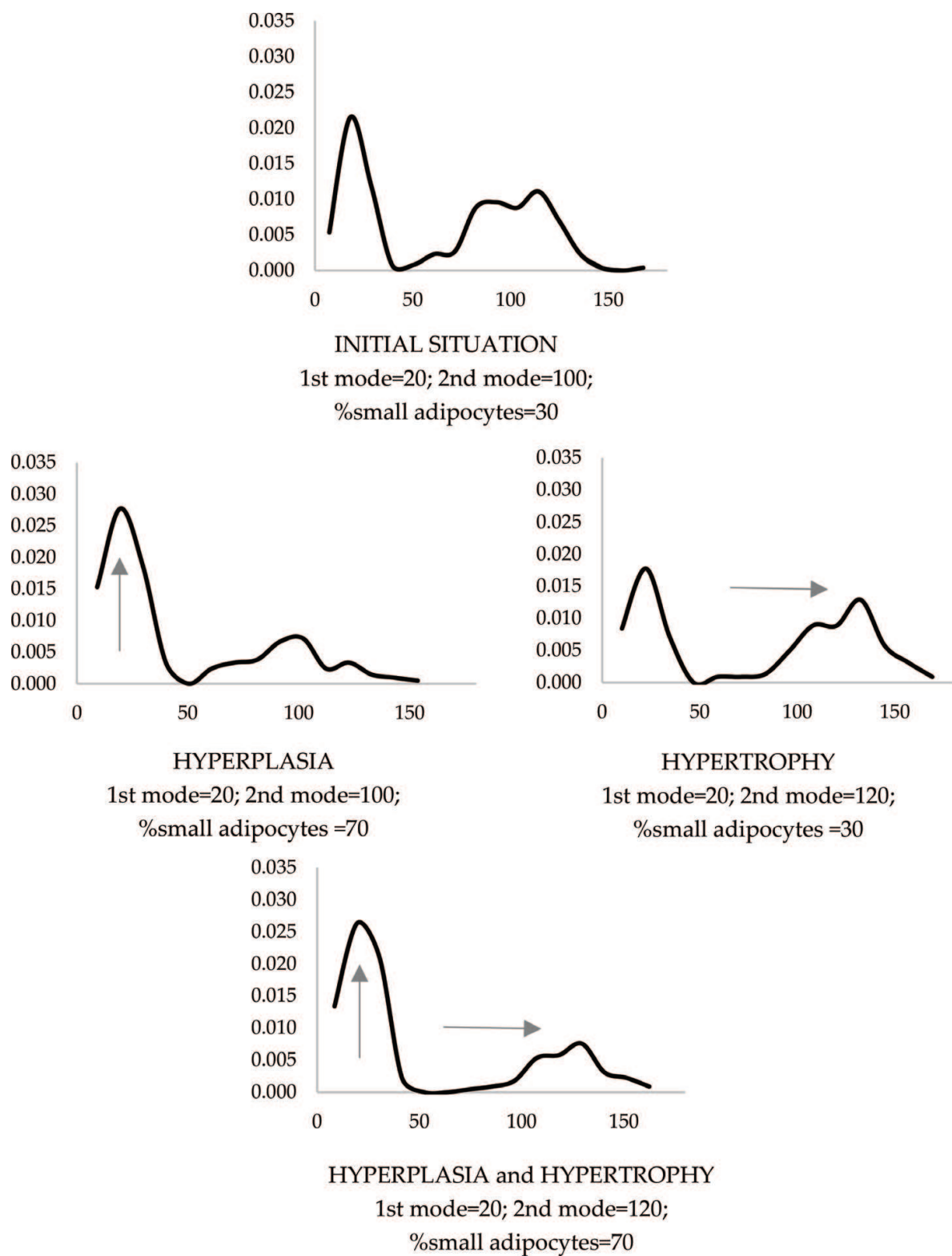
Figure 3. Simulated adipocyte size distribution depicting changes in the bimodal distributions in conditions of hyperplasia and hypertrophy. Arrows indicate the changes observed.

and normal distributions [80]. In any case, before putting any of these approaches to use, it is advisable first to check whether distributions are bimodal [81], since even exploratory data analysis methods like histograms may be misleading [82].

Alfonso and Mendizabal [81] proposed using the bimodality coefficient (BC) [83] and the dip statistic [84] to test the unimodal distribution of the data without having to assume any distribution underlying the bimodal distribution, though other approaches have also been described [85]. If the data prove not to be unimodal, the bimodal adipocyte size distribution can be described by estimating the two distribution modes and the proportion of adipocytes located under the inflexion point (nadir) between the two, thus enabling one population of small adipocytes to be differentiated from a second population of large ones. These three parameter values can be related to the degree of fatness in the animals as illustrated in **Figure 2**. The relationship needs not be unique but may vary with the species, tissue, age, physical growth, reserve mobilisation, etc. according to the relative importance of hyperplasia and hypertrophy that may be taking place at the time.

Figure 3 depicts what happens to these parameters with an increase in the number of small adipocytes (hyperplasia) or an increase in the size of large adipocytes (hypertrophy). This is a simulated example (as described by Alfonso [86]), which shows that the hypothesis of hyperplasia (an increase in the number of adipocytes) can be tested on the basis of the differences occurring in the percentage of small adipocytes and the hypothesis of hypertrophy (an increase in the diameter of large adipocytes) can be tested on the basis of the differences occurring in the second mode. The effect of both processes combined is also shown, since both changes in adipocyte number and changes in adipocyte size are known to take place simultaneously [87].

Analysing the data in this way allows differences between groups of animals to be described even in the presence of bimodality. Thus, differences between tissues in cattle [68] and pigs [81, 85], between breeds [85] and between animals grouped by age [88] and sex [85] have been described. Describing these differences is an essential basis for subsequently studying cell multiplication and differentiation both at the genetic level and at the level of expression, the better to explain the mechanisms of adipose tissue growth and be able to act appropriately so as to modulate them with a view to improving animal production systems. These same working methods can be extrapolated to the study of adipose tissue in humans and may help explain the factors involved in obesity and associated health issues.

6. Conclusion

Adipose tissue plays a fundamental role in maintaining the energy balance in animals, storing energy in the form of triglycerides during periods of high energy intake and releasing energy through lipolysis during periods of caloric restriction. In domesticated animals these triglycerides are located in different depots which vary in size and composition with species, sex, age and diet. These same factors in animal production systems condition adipose tissue growth brought about hyperplasia, or the proliferation of adipocytes, and hypertrophy, or the increase in adipocyte size. Both processes are regulated by different factors that stimulate or inhibit the expression of adipogenic and lipogenic genes. A knowledge of these processes enhances our

understanding of the genetic basis underlying the growth of fat depots in domesticated animals so as to be able to modify their quantity and composition. Thus, studying adipocyte size distributions helps us understand the different levels of involvement of, on the one hand, hypertrophy and hyperplasia and, on the other, of adipogenic and lipogenic genes and regulatory factors on adipose tissue growth in different groups of animals.

Acknowledgements

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