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COMPREHENSIVE REVIEWS

Current Methods of Adipogenic Differentiation of Mesenchymal Stem Cells

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There has been a recent increase in our understanding in the isolation, culture, and differentiation of mesenchymal stem cells (MSCs). Concomitantly, the availability of MSCs has increased, with cells now commercially available, including human MSCs from adipose tissue and bone marrow. Despite an increased understanding of MSC biology and an increase in their availability, standardization of techniques for adipogenic differentiation of MSCs is lacking. The following review will explore the variability in adipogenic differentiation in vitro, specifically in 3T3-L1 and primary MSCs derived from both adipose tissue and bone marrow. A review of alternative methods of adipogenic induction is also presented, including the use of specific peroxisome proliferator-activated receptor-gamma agonists as well as bone morphogenetic proteins. Finally, we define a standard, commonly used adipogenic differentiation medium in the hopes that this will be adopted for the future standardization of laboratory techniques—however, we also highlight the essentially arbitrary nature of this decision. With the current, rapid pace of electronic publications, it becomes imperative for standardization of such basic techniques so that interlaboratory results may be easily compared and interpreted.

Introduction

The adipogenic differentiation of mesenchymal stem cells (MSCs) and multipotent cell lines is of basic interest to many disparate specialties of medicine. Despite the growing body of research in obesity and adipose biology, MSC adipogenic differentiation is not restricted to endocrinologists. Stem cell scientists, bone biologists, and tissue engineering specialists all have a vested interest in the study of MSC adipogenesis. From a clinical standpoint, surgeons are faced with challenging reconstructive cases in patients afflicted with soft tissue resorption. For example, burn patients often suffer from soft tissue atrophy and would greatly benefit from soft tissue augmentation. Similarly, the wide use of highly active antiretroviral therapy medications in human immunodeficiency virus patients has left many patients with facial lipodystrophy, which can be socially troublesome. Such patients would greatly benefit from a tissue engineering approach to reconstruct their inadequate adipose compartment. With this wide variety of scientific backgrounds—from endocrinologists to stem cell biologists to surgeon-scientists—it stands to reason that the methods for adipogenic differentiation may also vary.

This potential variation in technique is only compounded by the commercial availability of MSCs derived from a variety of species and tissue types. Although the fact that one can order overnight as many viable human stem cells as desired is an amazing accomplishment of science, communication, and transportation, it brings with it several problems. For example, companies often send with their MSCs a proprietary medium whose contents are highly controlled but not reported to the customer. This practice is clearly driven by economic realities but not scientifically justified.

It is on the backdrop of these obvious shortcomings that this concise review article will explore the extreme variability in adipogenic differentiation medium between institutions, specifically looking at 3T3-L1 cells and primary MSCs of adipose tissue and bone marrow origin. Presumptively, the adipogenic supplements for a cell line (3T3-L1) would be highly controlled in comparison to primary cells; however, we found significant variability among both cell types. A review of alternative methods of adipogenic induction is also presented, including the use of specific peroxisome proliferator-activated receptor-gamma (PPARγ) agonists as well as bone morphogenetic proteins (BMPs). Finally, we define a standard, commonly used adipogenic differentiation medium for each cell type with the hopes that this may prompt a standardization of basic laboratory practices. Although the standardization of basic in vitro assays is necessary, there exists by no means a "quick-fix"

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for this dilemma. However, our hopes are that this and similar reviews will bring attention to this pressing scientific problem.

Materials and Methods

An exhaustive literature review was performed using PubMed. Search terms included “adipogenic differentiation 3T3-L1,” “bone marrow mesenchymal stem cell adipogenic differentiation,” “adipose stem cell adipogenic differentiation,” “rosiglitazone adipogenic differentiation,” and “bone morphogenetic protein adipogenic differentiation.” Results were stratified by species of origin, focusing only on those articles describing culture of either mouse or human cells. All articles in 2010 with full text available were examined. Finally, those companies that sell propriety adipogenic differentiation medium were contacted in the hopes that they would share their standardized recipes. No commercial entities were willing to share their medium components.

Results

Adipogenic differentiation of 3T3-L1 cells

3T3-L1 cells are the most commonly studied adipogenic cell line that is available through American Type Culture Collection (American Type Culture Collection No. CL-173). The L1 substrain of 3T3 was developed through clonal isolation. Generally, 3T3-L1 cells undergo adipogenic differentiation rapidly, within 1 week in most instances. In the last year, 45 articles have been published across United States, European, and Asian academic centers (Fig. 1A) [1–45]. As demonstrated in Fig. 1B, articles using 3T3-L1 cells have been published in a wide range of journals including *Biochemistry*, *Cell Biology*, *Endocrinology*, and others (Fig. 1B). The majority of articles use standard Dulbecco’s modified Eagle’s medium supplemented with 10% fetal bovine serum; however, this varies. Additional components are numerous; however, generally 3 components are used for nearly all differentiation of 3T3-L1 cells. These standardly include insulin, dexamethasone, and IBMX. For a cell line, all components were found to vary considerably in concentration from article to article (Fig. 1C). Insulin is widely used to induce proliferation and differentiation of preadipocytes [46]. At high concentrations, insulin is known to mimic insulin-like growth factor-1, activating mitogen-activated protein kinase pathways [47,48]. Dexamethasone is an anti-inflammatory steroid molecule that stimulates both osteogenic and adipogenic differentiation in a cell-, time-, and concentration-dependent manner [49,50]. However, when MSCs experience either prolonged exposure or increased concentrations of dexamethasone, they yield higher number of adipocytes in cultures while inhibiting osteogenic differentiation [51]. IBMX in combination with dexamethasone regulates PPARγ, promoting adipogenesis [52]. IBMX is a competitive, nonselective phosphodiesterase inhibitor, raising intracellular cAMP and protein kinase A (PKA). PKA signaling pathway is required for transcriptional activation of PPARγ and thus adipogenic gene expression [18]. In addition, both dexamethasone and IBMX are inducers of C/EBPδ and C/EBPβ, which are transcription factors for growth and differentiation [53]. Despite the consistent use of these 3 components, concentrations for each vary widely (Fig. 1C). For example, the concentration of insulin varies from 0 to 1,800 nM depending on the article. A list of additional components occasionally added, such as PPARγ agonists, is given in Table 1. A comprehensive breakdown of major components by article is available in Table 2.

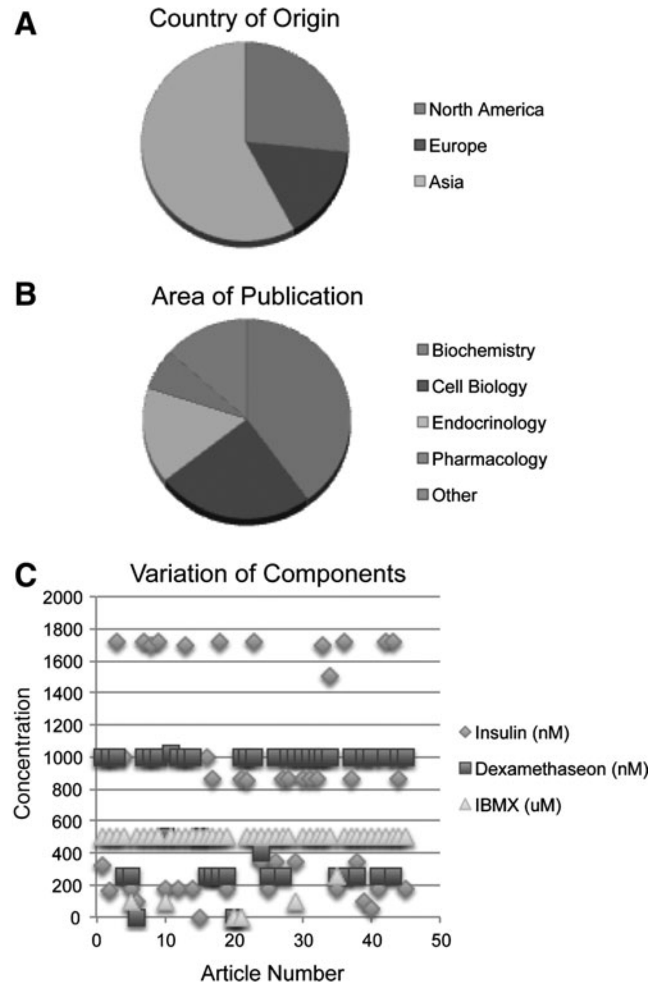

FIG. 1. Variation in adipogenic differentiation of 3T3-L1 cells. A literature review for articles within 2010 was performed for any publication examining the adipogenic differentiation of 3T3-L1 preadipocytes. **(A)** Breakdown of 45 publications by country of origin. **(B)** Breakdown of publications by area of scientific interest. **(C)** Breakdown of 3 major components of induction medium used for each individual publication. Although nearly all publications used insulin, dexamethasone, and IBMX, the concentrations varied widely. See Table 2 for a complete listing of induction components.

TABLE 1. ADDITIONAL COMPONENTS OF 3T3-L1 ADIPOGENIC INDUCTION MEDIUM

Rosiglitazone
Troglitazone
Biotin
Pantothenate
Triiodothyrone
Transferrin
Indomethacin
Hydrocortisone
Cortisol
Corticosterone

Table 2. Use of 3T3-L1 Cells

Article no.	PMID	Field	Country	FBS (%)	Insulin (nM)	Dexamethasone (nM)	IBMX (μM)
1	21161354	Pharmacology	Taiwan	10	320	1,000	500
2	21152033	Other	United States	10	167	1,000	500
3	21140438	Biochemistry	Canada	10	1,721	1,000	500
4	21136482	Cell biology	Canada	10	1,000	250	500
5	21084448	Endocrinology	Italy	10	175	250	100
6	21084441	Endocrinology	Germany	5	100	0	500
7	21080714	Biochemistry	Taiwan	10	1,720	1,000	500
8	21080334	Biochemistry	China	10	1,700	1,000	500
9	21053274	Biochemistry	The Netherlands	10	1,720	1,000	500
10	21047783	Biochemistry	Korea	10	172	500	100
11	21037091	Other	United States	10	12,052	1,020	500
12	21036149	Biochemistry	Japan	10	172	1,000	500
13	21031614	Other	Korea	10	1,700	1,000	500
14	20951125	Pharmacology	Korea	10	172	1,000	500
15	20943959	Cell biology	Canada	10	0.2	500	500
16	20881252	Endocrinology	Italy	10	1,000	250	500
17	20826223	Biochemistry	Korea	0	860	250	500
18	20719859	Endocrinology	Japan	10	1,720	250	500
19	20693579	Biochemistry	Korea	10	172	250	500
20	20665227	Other	Taiwan	10	0	0	0
21	20661713	Pharmacology	Korea	10	860	1,000	0
22	20656681	Biochemistry	Spain	10	850	1,000	500
23	20648622	Cell biology	China	10	1,720	1,000	500
24	20638365	Biochemistry	Korea	10	344	400	500
25	20627088	Biochemistry	Korea	10	172	250	500
26	20552250	Cell biology	Austria	10	344	1,000	500
27	20529675	Biochemistry	Korea	10	860	250	500
28	20519739	Cell biology	United States	10	860	1,000	500
29	20471953	Biochemistry	China	10	344	1,000	100
30	20460371	Biochemistry	United States	10	860	1,000	500
31	20444940	Endocrinology	China	10	860	1,000	500
32	20427709	Cell biology	China	10	860	1,000	500
33	20427485	Endocrinology	United States	10	1,700	1,000	500
34	20406654	Other	Taiwan	10	1,500	1,000	500
35	20357182	Cell biology	Korea	10	172	250	250
36	20346961	Cell biology	Korea	10	1,720	250	500
37	20200519	Other	United States	10	860	1,000	500
38	20181984	Cell biology	United States	10	344	250	500
39	20179325	Biochemistry	Canada	10	100	1,000	500
40	20133456	Endocrinology	Spain	10	50	1,000	500
41	20097210	Cell biology	Korea	10	172	250	500
42	20093363	Biochemistry	Japan	10	1,720	1,000	500
43	20081842	Cell biology	Japan	10	1,720	250	500
44	20060380	Biochemistry	Korea	10	860	1,000	500
45	20036887	Biochemistry	United States	10	172	1,000	500
Average				10	1,037.04	703.78	445.56
Median				10	860	1,000	500

FBS, fetal bovine serum.

Adipogenic differentiation of primary BMSCs

Unlike an immortalized cell line, primary cells are a heterogenous mixture of MSCs, unipotential and bipotential cells, and fibroblastic cells among numerous other cell types. With this variability in cell population, one would expect that the adipogenic differentiation of primary MSCs is, expectedly, even more variable (Fig. 2). For the purposes of this literature review, 2 of the most commonly studied MSC populations were examined: bone marrow mesenchymal stem cells (or BMSCs) and adipose-derived mesenchymal stem cells (most commonly abbreviated ASCs).

In the last year, ~20 articles have been published on the in vitro adipogenic differentiation of mouse and human BMSCs, respectively (see Tables 3 and 4 for a complete listing). The majority of these articles were published in the United States or Asia (Fig. 2A) and were primarily in the fields of cell biology and biochemistry (Fig. 2B). Generally, a 3-component cocktail is used for BMSC adipogenic induction, including insulin, dexamethasone, and IBMX (Fig. 2C). Vast inconsistency exists, however, between published protocols. Notice that a logarithmic scale is used for Fig. 2C, illustrating the extreme variability from 1 protocol to another. Moreover, a 1- or 2-component cocktail is sometimes

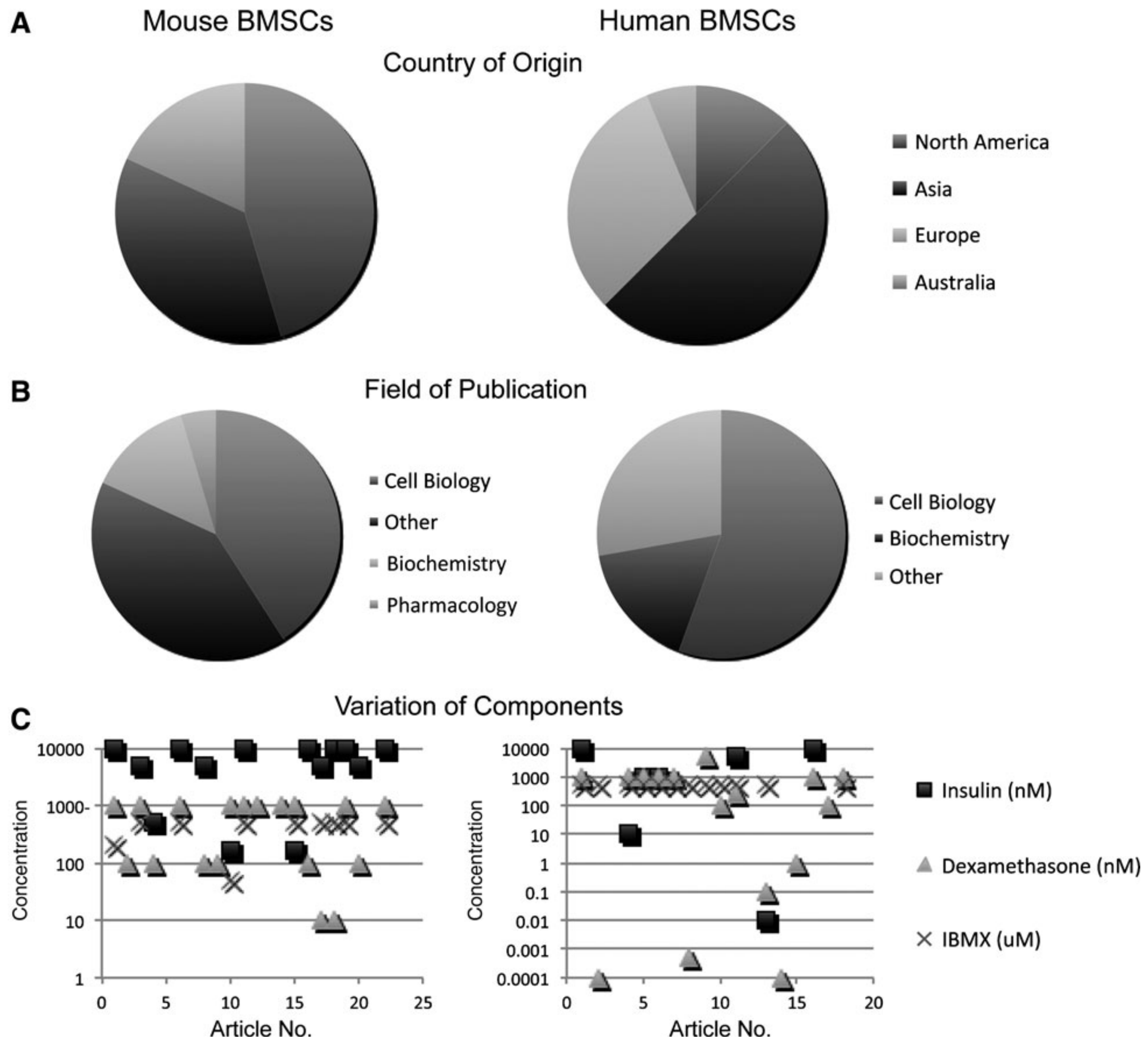

FIG. 2. Variation in adipogenic differentiation of BMSCs. Again, a literature review for articles within 2010 was performed for any publication examining the adipogenic differentiation of BMSCs—broken down by either mouse (*left*) or human (*right*) origin. **(A)** Country of origin for each article. **(B)** Area of scientific interest. **(C)** Breakdown of 3 major components of induction medium used for each individual publication. Although most publications used insulin, dexamethasone, and IBMX, the concentrations varied widely. See Tables 3 and 4 for a complete listing of induction components. BMSCs, bone marrow mesenchymal stem cells.

used, with the other drugs simply omitted (Fig. 2C). A comprehensive breakdown of major components by article and by species is available in Tables 3 and 4.

Interestingly, the concentrations of adipogenic components used for mouse and human BMSC culture differ significantly. Figure 3 demonstrates the clear difference in the standard concentration of supplements when taking into account species of derivation. Insulin was not used in the majority of publications in human BMSCs, whereas a near 100% increase in dexamethasone and a presence of indomethacin was observed in human compared with mouse BMSCs (Fig. 3). Collectively, these results suggest overall that a different stimulus may be needed for human compared with murine BMSC adipogenic differentiation.

Adipogenic differentiation of primary ASCs

In the last year, ∼20 articles have been published on the in vitro adipogenic differentiation of ASCs [22,54–79]. The majority of these articles were published in the United States (Fig. 4A) and were primarily in the fields of biochemistry and cell biology (Fig. 4B). Generally, a 3–4-component cocktail is used for ASC adipogenic induction, including indomethacin, insulin, dexamethasone, and IBMX (Fig. 3C). As with BMSCs, vast inconsistency exists between published protocols (Fig. 4C). A few articles use propriety, undisclosed components for adipogenic induction [75]. A comprehensive breakdown of major components by article is available in Tables 5 and 6. Interestingly, and in similarity to BMSCs, the

Table 3. Use of Mouse Bone Marrow Mesenchymal Stem Cells

Article no.	*PMID*	*Field*	*Country*	*FBS (%)*	*Insulin (nM)*	*Dexamethasone (nM)*	*Indomethacin (μM)*
1	19243475	Biology	China	10	10,000	1,000	200
2	20939016	Other	United States	10	10,000,000	100	0
3	20877012	Dentistry	Japan	10	5,000	1,000	0
4	20872592	Orthopedics	United States	9	500	100	50
5	20850355	Immunology	Japan	10	0	0	0
6	20692234	Biochemistry	Japan	10	10,000	1,000	0
7	20676132	Oncology	Japan	10	0	0	0
8	20672310	Biochemistry	United States	10	5,000	100	0
9	20649960	Other	United Kingdom	10	0	100	0
10	20506495	Biology	United States	10	167	1,000	0
11	20498072	Other	United States	10	10,000	1,000	0
12	20417614	Biology	Finland	10	50,000	1,000	0
13	20410440	Biology	United States	10	0	0	0
14	20374652	Biology	Finland	10	50,000	1,000	0
15	20374200	Biology	United States	0	167	1,000	0
16	20363288	Biology	China	10	10,000	100	0
17	20200977	Neuroscience	Australia	10	5,000	10	0
18	20039258	Orthopedics	United States	10	10,000	10	0
19	20007694	Biochemistry	United States	10	10,000	1,000	0
20	19929432	Pharmacology	Canada	10	5,000	100	0
21	20875915	Biology	Korea	10	0	0	0
22	20590530	Biology	Japan	0	10,000	1,000	0
Average				9	463,219.73	482.73	11.36
Median				10	5,000	100	0

differences in induction components between ASCs of mouse or human origin differ significantly (Fig. 3). For example, insulin concentration is approximately equivalent between mouse ASCs (mASCs) and human ASCs, whereas an ∼10-fold increase in dexamethasone concentration was observed in mASCs in comparison to their human counterpart (Fig. 3). These results again suggest clear differences in adipogenic induction between mouse and human MSCs.

Table 4. Use of Human Bone Marrow Mesenchymal Stem Cells

Article no.	*PMID*	*Field*	*Country*	*FBS (%)*	*Insulin (nM)*	*Dexamethasone (nM)*	*Indomethacin (μM)*
1	19243475	Biology	China	10	10,000	1,000	200
2	20939016	Other	United States	10	10,000,000	100	0
3	20877012	Dentistry	Japan	10	5,000	1,000	0
4	20872592	Orthopedics	United States	9	500	100	50
5	20850355	Immunology	Japan	10	0	0	0
6	20692234	Biochemistry	Japan	10	10,000	1,000	0
7	20676132	Oncology	Japan	10	0	0	0
8	20672310	Biochemistry	United States	10	5,000	100	0
9	20649960	Other	United Kingdom	10	0	100	0
10	20506495	Biology	United States	10	167	1,000	0
11	20498072	Other	United States	10	10,000	1,000	0
12	20417614	Biology	Finland	10	50,000	1,000	0
13	20410440	Biology	United States	10	0	0	0
14	20374652	Biology	Finland	10	50,000	1,000	0
15	20374200	Biology	United States	0	167	1,000	0
16	20363288	Biology	China	10	10,000	100	0
17	20200977	Neuroscience	Australia	10	5,000	10	0
18	20039258	Orthopedics	United States	10	10,000	10	0
19	20007694	Biochemistry	United States	10	10,000	1,000	0
20	19929432	Pharmacology	Canada	10	5,000	100	0
21	20875915	Biology	Korea	10	0	0	0
22	20590530	Biology	Japan	0	10,000	1,000	0
Average				9	463,219.73	482.73	11.36
Median				10	5,000	100	0

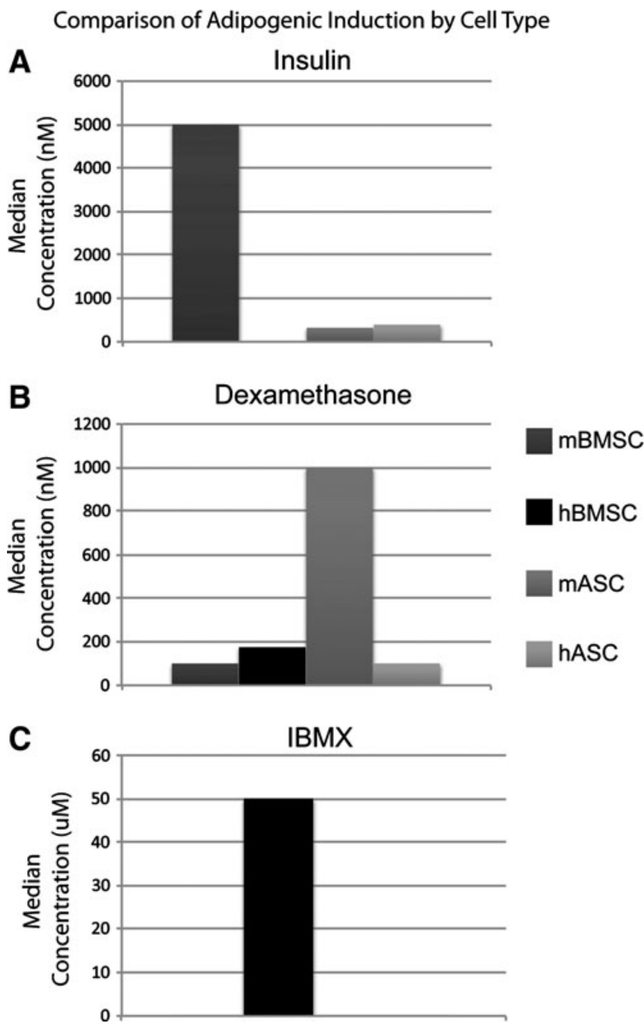

FIG. 3. Differences in adipogenic differentiation of BMSCs and ASCs based on species. Median values for each component of BMSC/ASC adipogenic induction medium was calculated and compared between mouse and human cells. **(A)** Mean concentration of insulin. **(B)** Mean concentration of dexamethasone. **(C)** Mean concentration of IBMX. ASCs, adipose-derived mesenchymal stem cells.

Use of PPARγ agonists

Other specialized components have been used to induce adipogenesis, either in addition to the standardized cocktail of agents or alone. One of the most commonly studied is the PPARγ agonist rosiglitazone, as well as similar agents (troglitazone, etc.). PPARγ agonists (thiazolidineldiones or glitazones) are not only a boon to those under treatment for diabetes but also, in general, work to speed up the differentiation of preadipocytes or adipoprogenitor cells in vitro. Rosiglitazone binds to PPARγ, thus "sensitizing" fat cells to insulin. It is known that glitazones reduce bone mineral density—postulated to be via diverting MSCs to adipogenesis rather than osteogenesis in vivo [80,81]. Glitazones are also known to increase bone loss via stimulation of osteoclasts and promotion of bone resorption [80]. There exists some debate as to the extent that rosiglitazone is able to induce MSC adipogenesis as a single agent [82]; however, in general, rosiglitazone both speeds and increases the degree of differentiation of adipoprogenitor cells. Thus, addition of rosiglitazone may be a useful addition to the standard adipogenic induction cocktail if cells are of late passage or otherwise resistant to speedy differentiation. A standard dose of 1 μM rosiglitazone is suggested.

Use of BMPs

BMPs are a subset of the transforming growth factor-β superfamily, so named as they were first observed to induce osteogenic differentiation when implanted in muscle pouch model. BMPs are powerful osteoinductive agents, and they have clear pleiotropic effects, including the induction of chondrogenesis [83], adipogenesis [84–86], and angiogenesis [87]. In some specific scenarios, the stimulation of BMP2 on adipogenesis results in the formation of cyst-like bone voids filled with lipid [88,89]. For example, in a recent study, BMP2 was implanted at high doses in a femoral defect in rats [90]. It was observed that there exists a dose-dependent increase in the formation of cyst-like bone voids with escalating doses of BMP2. Similar observations have been made in an ectopic bone formation model (nude mouse muscle pouch) by 2 independent investigators—in which various BMPs were observed to induce "lipid-laden" bone cysts [84,89]. BMP7 in particular (otherwise known as OP-1, which is also approved for human use for bone tissue regeneration) has been associated with adipogenic differentiation [91–93]. These observations bring up troubling questions regarding the lack of specificity of BMPs for skeletal tissue regeneration, but also whether BMPs may be appropriate induction agents for in vitro adipogenesis. In essence, should BMPs be standardly supplemented to adipogenic differentiation medium? BMP-induced adipogenesis, however, is a relatively newly described phenomenon and may have as-yet undescribed, potentially biologically relevant differences from so-called "standard" adipogenic differentiation. Thus, we would extend caution to those supplementing BMPs to "standardized" adipogenic medium, unless specifically studying this interesting phenomenon in cell signaling.

Discussion

In summary, while the use of in vitro adipogenic differentiation of MSCs has increased in recent years, a lack of clear standardization is clear from the present review. Overall, improved standardization of basic laboratory techniques such as adipogenic differentiation will vastly improve the interpublication comparability. In examining the averages and medians of adipogenic induction medium, we suggest the following formulas (see Tables 2–6 and 7 for a summary). Noteably, these are based on a compromise between all available techniques for the past year and not the authors' current laboratory practices.

For 3T3-L1 cells, 1,000 nM insulin, 700 nM dexamethasone, and 500 μM IBMX are used. For mBMSCs, 5,000 nM insulin and 100 nM dexamethasone are used. For hBMSCs, 175 nM dexamethasone and 50 μM indomethacin are used. For mASCs, 320 nM insulin and 1,000 nM dexamethasone are used. For human ASCs, 393 nM insulin and 100 nM dexamethasone are used. All induction components should include 10% fetal bovine serum and no other components unless specifically being tested. Although no single recipe is

Table 5. Use of Mouse Adipose-Derived Mesenchymal Stem Cells

Article no.	PMID	Field	Country	FBS (%)	Insulin (nM)	Dexamethasone (nM)	Indomethacin (μM)
1	21161354	Pharmacology	Taiwan	10	320	1,000	0
2	21152033	Other	United States	10	167	1,000	0
3	21140438	Biochemistry	Canada	10	1,721	1,000	0
4	21136482	Cell biology	Canada	10	1,000	250	0
5	21084448	Endocrinology	Italy	10	175	250	0
6	21084441	Endocrinology	Germany	5	100	0	0
7	21080714	Biochemistry	Taiwan	10	1,720	1,000	0
8	21053274	Biochemistry	The Netherlands	10	1,720	1,000	0
9	21047783	Biochemistry	Korea	10	172	500	0
10	21037091	Other	United States	10	12,052	1,020	0
11	21036149	Biochemistry	Japan		172	1,000	0
12	21031614	Other	Korea	10	1,700	1,000	0
13	20951125	Pharmacology	Korea	10	172	1,000	0
14	20943959	Cell biology	Canada	10	0.2	500	0
15	20881252	Endocrinology	Italy	10	1,000	250	0
16	20826223	Biochemistry	Korea	10	860	250	0
Average				10	1,422.08	688.75	0
Median				10	320	1,000	0

the definitive "cocktail" for adipogenic differentiation, we suggest these concentrations as a reasonable starting point for new experiments. Such attempts at standardization will improve interlaboratory comparisons.

At least for primary MSCs, a certain degree of heterogeneity in adipogenic induction supplements is understandable—as in fact there is still debate about the exact identity of MSCs. For example, the stem cell surface markers characteristic of ASCs are still being examined, and so a precise identity and purity of these cell populations are still forthcoming. Despite our evolving definition of an MSC, the clear lack of standardization of adipogenic differentiation is quite apparent based on our review. To this end, we propose the aforementioned standardized components, which is a compromise based on available studies. Importantly, these adipogenic differentiation conditions represent by no means an ideal or maximal stimulation condition, but rather a simple average of recently published articles. Thus, these values have an essential arbitrary nature to them and should be simply used as a "starting-off" point rather than a "gold standard."

Table 6. Use of Human Adipose-Derived Mesenchymal Stem Cells

Article no.	PMID	Field	Country	FBS (%)	Insulin (nM)	Dexamethasone (nM)	Indomethacin (μM)
1	21039998	Other	Korea	10	10,000,000	1,000	1
2	20932943	Bioengineering	United States	10	0	0	0
3	20807102	Biology	Israel	10	0	100	0
4	20709022	Biochemistry	Japan	10	0	1,000	0
5	20653721	Dermatology	Korea	10	1,000	1,000	200
6	20640914	Engineering	China	10	0	0	0
7	20601560	Surgery	Japan	0	0	0	0
8	19852056	Other	United States	4	10,000	1,000	60
9	20070733	Other	Korea	5	10,000	1,000	200
10	20304481	Other	Canada	0	66	0	0
11	20370354	Other	Germany	0	66	100	0
12	20380539	Other	United States	3	1,000	1,000	0
13	20420826	Other	United States	10	10,000	1,000	200
14	20528671	Other	United States	10	720	1,000	60
15	20572797	Other	Germany	10	0	0	0
16	20590410	Other	Korea	10	1,000	10,000	100
17	21039998	Other	Korea	10	1,000	1,000	1
18	19863253	Engineering	Japan	10	—	1	—
19	19929314	Pharmacology	Italy	10	—	0	—
20	20693579	Biochemistry	Korea	10	—	0	—
21	20561744	Other	United States	10	0	0	0
22	20097210	Biology	Korea	10	—	—	—
Average				8	557,491.8	914.33	45.67
Median				10	393	100	0

Table 7. Suggested Formulas for Adipogenic Differentiation

	FBS (%)	Insulin (nM)	Dexamethasone (nM)	Indomethacin (μM)	IBMX (μM)
Mouse BMSCs	10	5,000	100	0	0
Human BMSCs	10	0	175	50	0
Mouse ASCs	10	320	1,000	0	0
Human ASCs	10	393	100	0	0

ASCs, adipose-derived mesenchymal stem cells; BMSCs, bone marrow mesenchymal stem cells.

One additional surprising result from our study besides the clear interlaboratory variation was the difference in adipogenic stimuli used for mouse and human cells (Fig. 3). For example, a 10-fold difference in dexamethasone concentration was observed between mouse and human ASCs (Fig. 3B). Such a difference could be in part anticipated, as species of derivation seems to impart basic biologic differences onto ASCs. For example, we have previously observed that mouse and human ASCs differ significantly in their ability to differentiate down an osteogenic lineage, both in vitro and in vivo [94–96]. In addition, cytokine responsiveness seems to differ as well. For example, transforming growth factor-β1

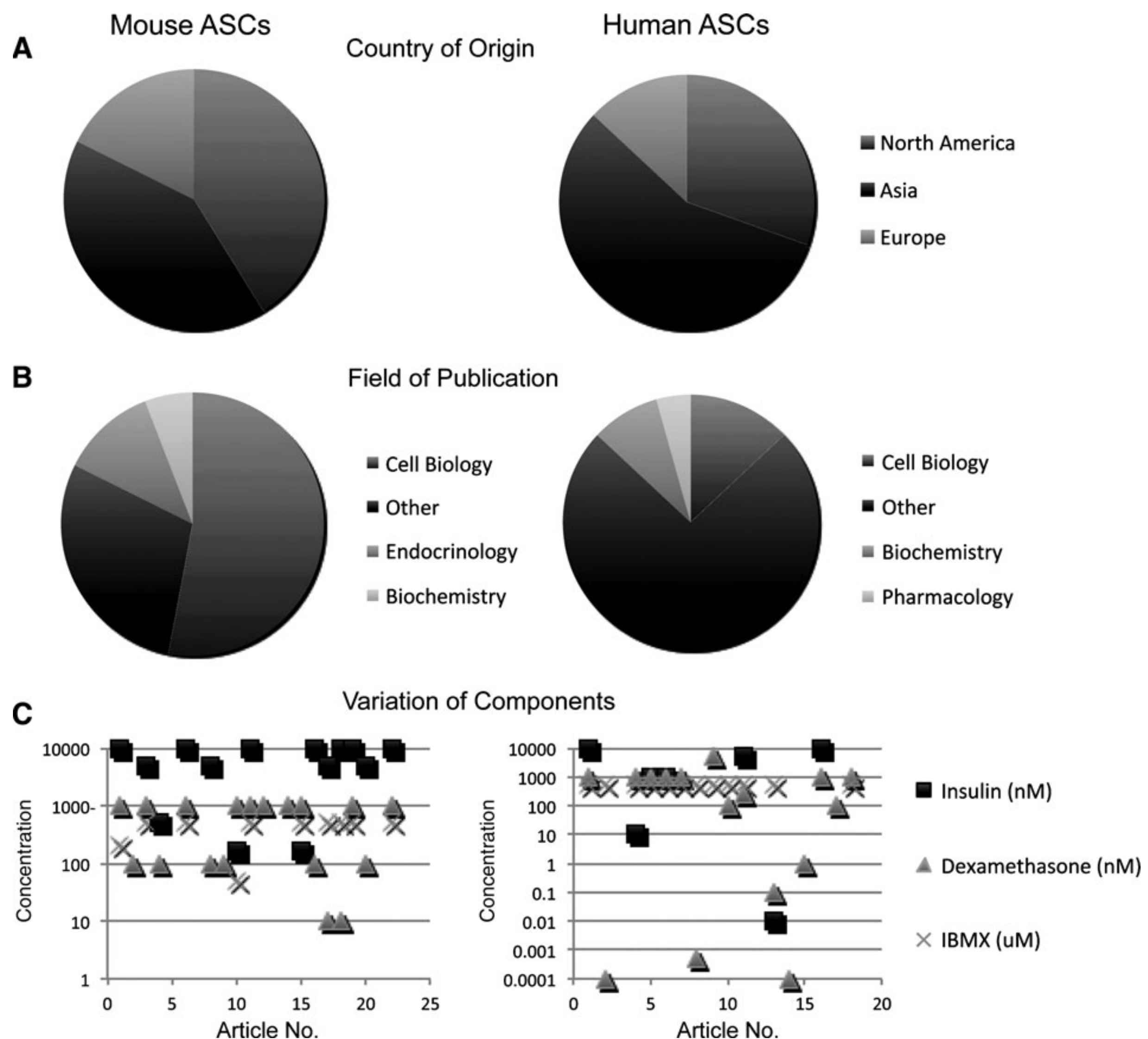

FIG. 4. Variation in adipogenic differentiation of ASCs. Again, a literature review for articles within 2010 was performed for any publication examining the adipogenic differentiation of ASCs—broken down by either mouse (*left*) or human (*right*) origin. **(A)** Country of origin. **(B)** Area of scientific interest. **(C)** Breakdown of 4 major components of induction medium used for each individual publication. Although most publications used indomethacin, insulin, dexamethasone, and IBMX, the concentrations varied widely.

appears to repress osteogenic differentiation in mouse ASCs; however, it has a significantly muted effect among human cells [96]. Such observations are indeed curious, and the basic interspecies differences among MSC populations have yet to be identified.

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Author Disclosure Statement

The authors have no conflicts of interest.

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