ISSN 1948-0210 (online)

World Journal of *Stem Cells*

World J Stem Cells 2022 October 26; 14(10): 744-776

Published by Baishideng Publishing Group Inc

WJSC

World Journal of Stem Cells

Contents

Monthly Volume 14 Number 10 October 26, 2022

MINIREVIEWS

ORIGINAL ARTICLE

Basic Study

World Journal of Stem Cells

Monthly Volume 14 Number 10 October 26, 2022

Contents

NAME OF JOURNAL
World Journal of Stem Cells

ISSN
ISSN 1948-0210 (online)

LAUNCH DATE
December 31, 2009

FREQUENCY
Monthly

EDITORS-IN-CHIEF
Shengwen Calvin Li, Carlo Ventura

EDITORIAL BOARD MEMBERS
https://www.wjgnet.com/1948-0210/editorialboard.htm

PUBLICATION DATE
October 26, 2022

INSTRUCTIONS TO AUTHORS
https://www.wjgnet.com/bpg/gerinfo/204

GUIDELINES FOR ETHICS DOCUMENTS
https://www.wjgnet.com/bpg/GerInfo/287

GUIDELINES FOR NON-NATIVE SPEAKERS OF ENGLISH
https://www.wjgnet.com/bpg/gerinfo/240

PUBLICATION ETHICS
https://www.wjgnet.com/bpg/GerInfo/288

PUBLICATION MISCONDUCT
https://www.wjgnet.com/bpg/gerinfo/208

ARTICLE PROCESSING CHARGE
https://www.wjgnet.com/bpg/gerinfo/242

STEPS FOR SUBMITTING MANUSCRIPTS
https://www.wjgnet.com/bpg/GerInfo/239

ONLINE SUBMISSION
https://www.f6publishing.com

World Journal of Stem Cells

Submit a Manuscript: https://www.f6publishing.com

World J Stem Cells 2022 October 26; 14(10): 744-755

DOI: 10.4252/wjsc.v14.i10.744

ISSN 1948-0210 (online)

MINIREVIEWS

Therapeutic applications of adipose-derived stromal vascular fractions in osteoarthritis

Qi Tang, Xian-Sheng Zhao, Ao Guo, Ruo-Tong Cui, Huai-Le Song, Zi-Yang Qi, Yi Pan, Yue Yang, Fang-Fang Zhang, Liang Jin

Specialty type: Cell biology

Provenance and peer review: Unsolicited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's scientific quality classification

Grade A (Excellent): 0
Grade B (Very good): B, B
Grade C (Good): C, C
Grade D (Fair): D, D
Grade E (Poor): 0

P-Reviewer: Georgiev T, Bulgaria; Jennane R, France; Konrads C, Germany; Moretti A, Italy; Muthu S, India

Received: April 6, 2022
Peer-review started: April 6, 2022
First decision: June 22, 2022
Revised: July 8, 2022
Accepted: September 12, 2022
Article in press: September 12, 2022
Published online: October 26, 2022

Qi Tang, Ao Guo, Ruo-Tong Cui, Huai-Le Song, Zi-Yang Qi, Yi Pan, Yue Yang, Fang-Fang Zhang, Liang Jin, School of Life Science and Technology, China Pharmaceutical University, Nanjing 211198, Jiangsu Province, China

Xian-Sheng Zhao, Department of Dermatology, Huashan Hospital, Fudan University, Shanghai 200040, China

Corresponding author: Liang Jin, PhD, Professor, School of Life Science and Technology, China Pharmaceutical University, No. 24 Tongjiaxiang Avenue, Nanjing 211198, Jiangsu Province, China. ljstemcell@cpu.edu.cn

Abstract

Osteoarthritis (OA) is considered to be a highly heterogeneous disease with progressive cartilage loss, subchondral bone remodeling, and low-grade inflammation. It is one of the world's leading causes of disability. Most conventional clinical treatments for OA are palliative drugs, which cannot fundamentally cure this disease. The stromal vascular fraction (SVF) from adipose tissues is a heterogeneous cell population. According to previous studies, it contains a large number of mesenchymal stem cells, which have been used to treat OA with good therapeutic results. This safe, simple, and effective therapy is expected to be applied and promoted in the future. In this paper, the detailed pathogenesis, diagnosis, and current clinical treatments for OA are introduced. Then, clinical studies and the therapeutic mechanism of SVF for the treatment of OA are summarized.

Key Words: Arthritis; Articular cartilage; Stromal vascular fraction; Mesenchymal stem cells; Cell therapy

Core Tip: Osteoarthritis (OA) is one of the world's leading causes of disability. Clinically, palliative drugs cannot fundamentally cure this disease. The stromal vascular fraction (SVF) from adipose tissues is a heterogeneous cell population. According to studies, it contains a large number of mesenchymal stem cells, which have been used to treat OA with good therapeutic effects. In this review, we present an updated status of the comprehensive and systematic review of pathogenesis, diagnosis, and current clinical treatments for OA, especially focusing on therapeutic applications of adipose-derived SVF.

Citation: Tang Q, Zhao XS, Guo A, Cui RT, Song HL, Qi ZY, Pan Y, Yang Y, Zhang FF, Jin L. Therapeutic applications of adipose-derived stromal vascular fractions in osteoarthritis. *World J Stem Cells* 2022; 14(10): 744-755
URL: https://www.wjgnet.com/1948-0210/full/v14/i10/744.htm
DOI: https://dx.doi.org/10.4252/wjsc.v14.i10.744

INTRODUCTION

Osteoarthritis (OA) was once thought to be a degenerative joint disease, but recent research has revealed that cartilage, subchondral bone, and synovium in the joints can all develop and form varying degrees of inflammation. As a result, OA is considered a whole joint disease. It is marked by an imbalance in the catabolic and anabolic processes of articular cartilage, as well as compensatory changes in bone and synovial inflammation, all of which ultimately lead to joint dysfunction[1,2].

The major feature of OA is the degeneration of articular cartilage and subchondral bone, which eventually results in joint swelling, pain, stiffness, limited mobility, effusion and inflammation, and even disability. This disease not only causes patients to experience considerable pain but also places a great economic burden on them and on society. Aging, genetic susceptibility, obesity, high bone mineral density (BMD), joint overuse, and injury are among the main causes of OA, according to researchers[3].

The Global Burden of Disease study for 2017 was published in The Lancet in 2018, and Safiri used it to conduct a systematic analysis of OA. From 1990 to 2017, the global age-standardized point prevalence, annual incidence, and years lived with disability rates of OA increased by 9.3% (95% UI: 8%-10.7%), 8.2% (95% UI: 7.1%-9.4%), and 9.6% (95% UI: 8.3%-11.1%), respectively. It is worth noting that the prevalence of OA in females was higher than that in males. Furthermore, both the morbidity and prevalence of OA rise with age, with the prevalence of cases peaking at 60-64 years old[4].

Obesity, in addition to age, is a significant risk factor for OA. Obesity puts more strain on weight-bearing joints. At the same time, adipocytes may also play a role in the pathogenesis of OA by secreting inflammatory factors. These factors increase the production of matrix metalloproteinases (MMPs) and prostaglandins while inhibiting the synthesis of proteoglycans and type II collagen, affecting cartilage function[5]. Obese patients (BMI > 30 kg/m^2) are more likely to need total knee arthroplasty and have higher rates of postoperative complications, according to studies[6].

Genetic factors account for 30%-65% of OA cases. A genome-wide association scanning study has identified 21 independent OA susceptibility loci[7]. Articular cartilage injury, anterior cruciate ligament (ACL) rupture, and meniscus tear (MT) all greatly increase the risk of OA. According to one study, people who have had knee injuries are four times more likely to develop OA than those who have not had knee injuries[8]. In addition, a low vitamin D diet[9] and excessive joint use[10] may also increase the risk of OA. High BMD increases the risk of knee OA and joint space narrowing, but does not worsen the imaging progression of existing knee OA, according to one study[11].

Since OA can cause great personal and economic losses, we must pay attention to it. The purpose of this paper is to introduce the pathogenesis, diagnosis and current clinical treatment methods of OA and briefly summarize the clinical researches and mechanisms of SVF in the treatment of osteoarthritis.

PATHOGENESIS OF OA

Cartilage destruction, subchondral bone remodeling, osteophyte formation, and joint capsule thickening are all parts of the pathogenesis of OA, which may take several years. It should be noted that prearthritic deformities of the limbs could also lead to osteoarthritis. For example, severe valgus or varus malalignment leads to lateral or medial gonarthritis. This can produce a change in the distribution of high stresses within the joint, resulting in a force imbalance, which in turn accelerates arthritic pathology[12]. The inflammation of OA is low-grade and chronic, causing both the innate and adaptive immune systems to be activated (primarily the innate immune system), distinguishing OA from rheumatoid arthritis[13].

Normally, articular cartilage consists of water, chondrocytes, and extracellular matrices. The extracellular matrix is synthesized and secreted by chondrocytes and contains collagen (mainly type II collagen), proteoglycans, hyaluronic acid (HA), and small-molecule glycoproteins. Type II collagen and other extracellular matrices can form a reticular structure, which provides pressure resistance for articular cartilage[14]. When OA occurs, chondrocytes produce a variety of inflammatory cytokines and chemokines that cause inflammation, such as interleukin-1β (IL-1β), tumor necrosis factor-α (TNF-α), and interleukin-6 (IL-6). Concurrently, chondrocytes produce MMPs, as well as disintegrin and metalloproteinase with thrombospondin-like motifs (ADAMTS). Type II collagen is the target of disintegrin, and proteoglycans are the targets of ADAMTS; together, these proteins eventually lead to increased cartilage catabolism and reduced synthetic metabolism and repair[15]. It is worth noting that osteoblasts, synovial cells, and monocytes in joints can produce IL-1β, TNF-α, MMPs, *etc*[15].

Subchondral bone, which is located beneath the calcified layer of articular cartilage and contains blood vessels and nerves, may provide mechanical support for articular cartilage. In the early stage of OA, osteoclasts mediate the remodeling of subchondral bones. Magnetic resonance imaging (MRI) can detect various degrees of subchondral bone sclerosis, osteophytes, and subchondral bone cysts as OA progresses[16,17]. This modification may reduce the mechanical support provided by subchondral bones to articular cartilage, making the articular cartilage vulnerable to damage.

Synovitis is one of the most common complications of OA. The synovium may proliferate significantly during the progression of OA, and synovial cells may also secrete proinflammatory factors and MMPs to aid in the development of OA[18]. In addition to the local inflammatory mechanism of joints, increasing evidence suggests that obesity can keep the human body in a low-degree inflammatory environment for an extended period of time. Obese people have a significantly higher risk of OA than people with a normal BMI[6].

DIAGNOSIS AND THERAPY OF OA

The primary method of diagnosing OA is imaging examination, which mainly includes X-rays, computed tomography (CT), MRI, ultrasonography, and arthroscopy. X-ray examination is one of the preferred methods for the clinical diagnosis of OA[19,20]. It can detect key features of OA, such as joint space narrowing, osteophytes, subchondral bone sclerosis, and cysts. However, because X-ray images can only visualize cartilage damage indirectly by observing changes in joint spaces and bones, it cannot be used as the sole criterion for OA diagnosis, and it is not suitable for OA diagnosis in the early stages [21]. CT scan can reveal subtle bone changes in the subchondral bone. The images can clearly show the loose bodies formed by osteophyte shedding around the joints on different sides. As a result, it is more sensitive for detecting osteophytes and subchondral cysts[22]. Because MRI has higher tissue resolution, it can provide information about the size and structural integrity of cartilage, as well as directly show articular cartilage damage and changes in thickness[23]. Furthermore, because MRI can detect OA-inducing factors such as injuries to the ACL and meniscus, it is useful for detecting OA at an early stage [23]. Although ultrasonography can detect joint exudation and inflammation, it cannot diagnose deeper joints as well as MRI[24]. Arthroscopy is the use of an arthroscope to penetrate deep into the joint cavity and directly observe tissues such as the articular cartilage and meniscus. It can not only pinpoint the location of synovitis but can also clean up the joints by removing loose bodies, removing torn meniscus tissue, and trimming bone surfaces. This diagnostic method can both diagnose and treat OA, making it the most promising diagnostic tool for assessing joint damage[25]. When features of OA appear on images in the clinic, it usually means that the patient has irreversible cartilage damage.

Biomarkers have won a place in the diagnosis of OA because they can be used as indicators for its early diagnosis and prognosis. Biomarkers of OA are distributed in the blood, urine, and joint fluid and primarily consist of cytokines related to joint metabolism, extracellular matrix components, inflammatory factors, *etc.* Currently, commonly used indicators are cartilage oligomeric matrix protein, HA, proteoglycan, MMP, C-telopeptide of type II collagen (CTX-II), IL-6, and lactate dehydrogenase[26]. Although biomarkers can be used to make an early diagnosis of OA, their sensitivity and specificity are not satisfactory[27]. Because each of the methods listed above has advantages and disadvantages, it is necessary to combine them to provide comprehensive and reliable information for the diagnosis of OA, as well as a scientific reference for developing an OA treatment plan.

Treatment for OA primarily consists of lifestyle changes, physical therapy, drug therapy, intra-articular therapy, and surgery. However, there is no complete cure for OA. The goal of treatment in the early stage of OA is to reduce pain and stiffness. Therapeutic approaches normally involve moderate exercise, diet, and oral medication. The goal of treatment in the intermediate and late stages of OA is to maintain physical function. The therapeutic approaches normally include intraarticular injection therapy and surgery. According to the Osteoarthritis Research Society International, suitable structured land-based exercise is one of the most effective ways to treat OA, as it can reduce joint pain and stiffness. Furthermore, losing weight is also one of the important ways for obese patients to improve OA [28]. Physical therapy includes electrotherapy, thermotherapy, and acupuncture. Analgesic, anti-inflammatory, and cartilage-protecting drugs are commonly used in drug therapy. However, these therapies

can only alleviate pain symptoms and slow the inflammatory process; they cannot prevent the development of OA. In clinical practice, articular cavity injection therapy is a minimally invasive treatment method. The injected treatment agents include glucocorticoids, sodium hyaluronate, platelet-rich plasma (PRP), bone morphogenetic protein 7 (BMP-7), and stem cells. It should be noted that ultrasound can be used to guide the use of intraarticular injections in patients with advanced osteoarthritis and obesity. A novel intraarticular injectable drug-loaded delivery system using nanoscale materials, including micelles, liposomes, and dendrimers, has been proposed. This method can slow drug release and prolong drug retention time in the joint cavity[29]. The surgical therapies for OA treatment mainly include arthroscopic debridement, microfracture, allogeneic/autologous cartilage transplantation, and artificial joint replacement. For example, for frontal malalignment and varus or valgus malalignment, high tibial osteotomy could be used to correct the proximal tibial angle, thereby reducing pain and delaying the progression of arthritis[30]. However, surgery is invasive and comes with certain risks. Therefore, it is only applicable for patients who have severe degenerative changes in their joints, such as reduced or eliminated joint space, which severely impairs their quality of life[31]. This therapy is not appropriate for the treatment of early-stage OA.

STROMAL VASCULAR FRACTION

SVF is a multicellular component extracted from adipose tissues through a process that includes collagenase digestion, centrifugation, washing, and filtration. Clinically, liposuction, for example, can be used to obtain adipose tissues from areas such as the abdomen, groin, forearm, and hip. Methods currently used to extract SVF mainly include enzyme digestion and mechanical separation. Because the cell yield obtained by enzyme digestion is higher than that obtained by mechanical separation, researchers prefer enzyme digestion[32-34]. However, if the final product of the enzyme digestion method is not completely washed, it may retain exogenous collagenase, so it is subject to strict regulatory standards in use.

The cell components of SVF could be identified by cell surface molecules such as the cluster of differentiation (CD)[35]. According to the International Federation for Adipose Therapeutics and Science and the International Society for Cellular Therapy, SVF is primarily composed of adipose mesenchymal stem cells (ADSCs), hematopoietic stem/progenitor cells, lymphocytes, monocytes/macrophages, endothelial cells, and pericytes[36]. According to research, CD45-CD235a-CD31-CD34+ is a marker combination to identify SVF cells. ADSCs are a critical component of SVF, accounting for approximately 10% of the SVF cell population[37]. ADSCs endow SVF with characteristics of stem cells, and other cells also play a positive role in secreting active cytokines and regulating body immunity. Because SVF has demonstrated excellent therapeutic value in tissue regeneration, vascular reconstruction, and anti-inflammatory properties, it is widely used in clinical practice.

The keywords "stromal vascular fraction" and "osteoarthritis" were used to search the clinical research database (https://www.clinicaltrials.gov/). After the withdrawn studies were removed, the current clinical trial studies of SVF in the treatment of osteoarthritis were obtained, as shown in Table 1.

CLINICAL RESEARCHES OF SVF IN OA

Clinically, drug therapy for OA can relieve pain and some symptoms, but it cannot repair or inhibit the damage to cartilage and other joint tissues. As a result, it can neither fundamentally solve the problem of cartilage degeneration nor prevent the progression of OA. Although surgical therapy may be used to repair cartilage damage, the long-term treatment effect of OA is limited, and there are numerous drawbacks and risks. Additionally, it should be noted that surgical treatment is only suitable for the treatment of patients with OA in the middle and late stages. Therefore, the minimally invasive, safe, and rapid therapy of stem cell injection into the joint cavity has gained more attention and use.

Mesenchymal stem cells (MSCs) are derived from the mesoderm and can differentiate into adipocytes, osteoblasts, chondrocytes, and other mesoderm cells[38]. This differentiation potential makes MSCs a promising alternative treatment for OA. MSCs can be derived from the umbilical cord, bone marrow, and adipose tissue. When compared to other source tissues, adipose tissues are rich in content, easier to obtain (through liposuction), and contain more stem cells (ADSCs)[39]. ADSCs are a type of MSC. Furthermore, ADSCs have higher genetic stability, a higher ability for proliferation and differentiation, a lower rate of aging, and longer telomere length[40], making them the most promising treatment option for OA.

ADSCs can be obtained by SVF after *in vitro* adherence culture and amplification. However, because the cumbersome and time-consuming *in vitro* amplification step poses a contamination risk, as well as time and economic costs, the researchers shifted their focus to SVF, which does not require culture. The rich cellular components in SVF can not only differentiate into chondrocytes to aid in tissue repair but also secrete active cytokines and regulate the body's immune system to exert anti-inflammatory effects, among other things. As a result, clinical researchers prefer SVF.

Table 1 Clinical trials of stromal vascular fraction in the treatment of osteoarthritis

Main conditions	NCT number	Phase
Osteoarthritis	NCT03818737	Phase III
Osteoarthritis	NCT02846675, NCT02967874	Phase II
Knee osteoarthritis	NCT04050111	Phase II
Knee osteoarthritis	NCT02276833, NCT04043819, NCT03940950	Phase I
		Phase I
Osteoarthritis	NCT03166410, NCT02697682, NCT02726945	Preclinical
		Preclinical
Knee osteoarthritis	NCT04440189, NCT02726945, NCT04440189	Preclinical
		Preclinical

NCT: National Clinical Trial.

This study used the keywords "stromal vascular fraction" and "osteoarthritis" to search PubMed, Web of Science, Cochrane Library, and Google Scholar, and after removing duplicates and non-English literature, we screened clinical trials of SVF treatment for OA published between 2016 and 2020. Finally, 22 studies were obtained, as shown in Table 2[41-62].

The majority of the studies in Table 2 are for knee OA, and approximately half of the clinical studies are uncontrolled and without combination therapy, with only SVF being used to treat OA. No serious adverse reactions, such as infection, acute pain, or cancer, were reported in any of these studies, indicating that SVF treatment for OA is safe. Most studies used adipose tissue from the abdomen, buttocks, and lateral thigh, with a volume (for one knee) ranging from 60 to 215 mL; the number of SVF cells varied from 2×10^6 to 3×10^7; and the final volume of SVF preparation used for intra-articular injection varied from 2.5 to 5 mL. Although it is difficult to maintain a unified standard for the degree of OA, the volume of adipose tissues, and the number and viability of SVF cells, existing studies have demonstrated a positive therapeutic effect, with VAS, WOMAC, ROM, MRI, KOOS, Lysholm, and other scores improving, the patient's pain decreasing, and inflammation symptoms decreasing. Simultaneously, joint function has been improved to a certain extent[43,44,47-55,60-62]. Figure 1 depicts the general steps of using SVF to treat OA in a current clinical setting.

It is worth noting that Garza found a significant difference in the final treatment effect of OA patients between the high-dose SVF (3×10^7) and low-dose SVF (1.5×10^7) groups. They pointed out that the median WOMAC score changes in the two groups after 6 mo of treatment were 83.9% and 51.5%, respectively. At the same time, the WOMAC scores of the high-dose and low-dose groups differed significantly from that of the control group, indicating that the degree of OA improvement is SVF dose-dependent[60].

Interestingly, Michalek used collagenase digestion and mechanical methods to obtain SVF. The results showed that the cell yield of the former was 3.4 times higher than that of the latter, and the cell activity was similar. The researchers then used the obtained SVF to randomly treat OA patients, and they discovered that the SVF obtained by the two extraction methods had the same therapeutic effect on OA, with no significant difference. As a result, it can be concluded that the SVF extraction method has little effect on OA treatment[47].

THERAPEUTIC MECHANISMS OF SVF IN OA TREATMENT

When the body is damaged, signaling molecules are produced in the damaged area, activating the homing effect of stem cells, causing them to migrate to the damaged location and play a role in tissue repair[63]. However, cartilage tissues lack blood vessels. Even for bone marrow MSCs, it is difficult to migrate to the injury, so exogenous stem cell injection is needed. After SVF is injected into the joint cavity of patients, the ADSCs in the SVF can migrate to the damaged area through the interaction of various chemokine receptors (such as CXCR4, integrin, selectin, and vascular cell adhesion molecule-1)[64].

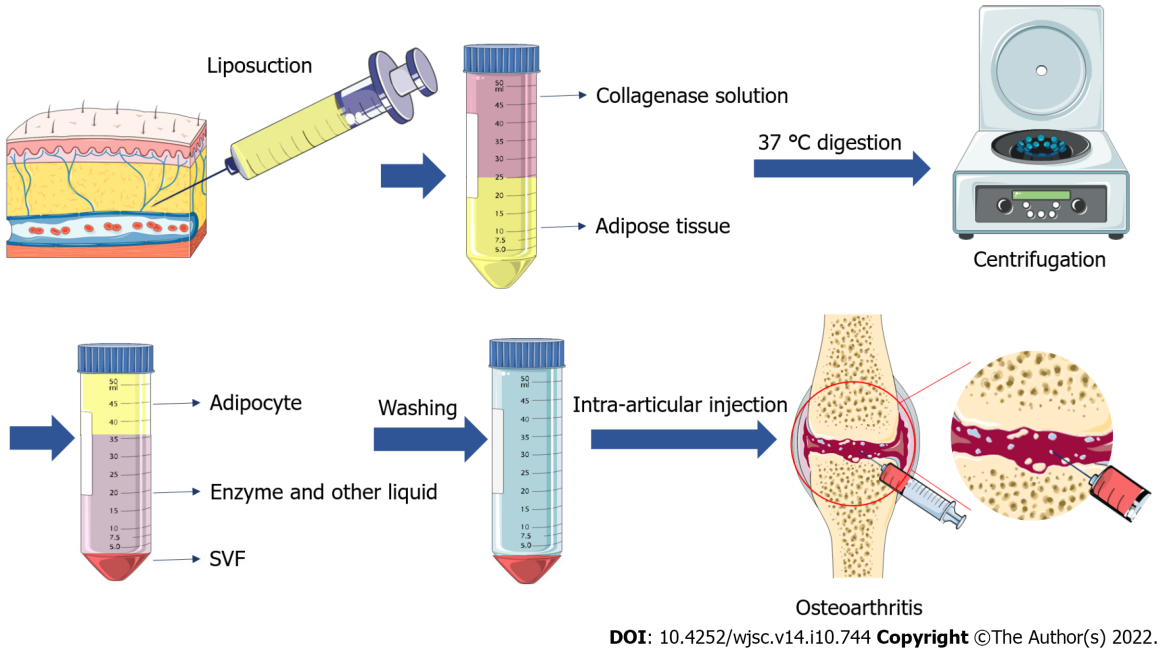
Although numerous studies have demonstrated that ADSCs can be induced to differentiate into chondrocytes in vitro, there is insufficient evidence to prove that ADSCs can differentiate into chondrocytes *in vivo* to repair damaged cartilage tissues during OA treatment. Existing evidence indicates that an important mechanism of the therapeutic effect of ADSCs is the nourishing effect of ADSCs; they can secrete growth factors and cytokines, such as transforming growth factor-β (TGF-β),

Table 2 Clinical researches on the treatment of osteoarthritis with stromal vascular fraction

Ref.	Treatment	Study type OA position/number	Patients	Follow-up time	Outcome assessments	Consequences
Pak *et al*[41,42] (2016, 2018)	SVF + HA + PRP	Case report Knee	3 patients: 1 male, age: 68; 2 females, age: 60 and 87	3.5 mo	FRI, MRI, ROM, VAS	Cartilage repaired showed by MRI; all scores improved
Fodor *et al*[43] (2016) NCT02357485	SVF	Pilot study Knee/8 OA	6 patients: 1 male, 5 females, mean age: 59	12 mo	ROM, WOMAC, VAS, TUG, MRI	All scores improved, no MRI evidence of cartilage regeneration
Yokota *et al*[44] (2017)	SVF	Case report Knee/26 OA	13 patients: 2 males, 11 females, mean age: 74.5	6 mo	VAS, JKOM, WOMAC	All scores improved
Nguyen *et al*[45] (2017) NCT02142842	AM *vs* AM + SVF + PRP	Comparative study Knee	30 patients (15 per group: 3 males, 12 females) mean age: 58	18 mo	VAS, Lysholm, WOMAC, MRI	All scores improved compared with AM group; AM + SVF + PRP group had obvious cartilage repair show by MRI
Michalek *et al* [46] (2017)	SVF	Case control multi-centric non-randomized study Knee and hip/1856 OA	1128 patients: 596 males, 532 females, median age: 62	17.2 mo	Modified KOOS/HOOS	KOOS/HOOS improved, most patients gradually improved, obesity and more severe OA healed slowly
Tantuway *et al* [47] (2017)	SVF	Case report Knee/201 OA	101 patients: 41 males, 60 females, age: 29-84	3-24 mo	KOOS	KOOS and joint function improved, pain relieved, patients could move normally
Russo *et al*[48, 49] (2017, 2018)	SVF	Retrospective study Diffuse degenerative keen	30 patients: 21 males, 9 females, median age: 43	12-36 mo	Lysholm, VAS, IKDC-subjective, KOOS	41%, 55%, 55%, 64% of the patients improved in the scores, respectively
Bright *et al*[50] (2018)	SVF	Case report Knee and hip	1 patient: female, age: 27	3 yr	WOMAC, HOOS	Symptoms of OA reduced, all scores improved, ankylosing spondylitis, depression, anxiety and fatigue improved
Barfod *et al*[51] (2019) NCT02697682	SVF	Prospective cohort study Knee	20 patients, mean age: 49	12 mo	KOOS	KOOS improved
Roato *et al*[52] (2019)	SVF	Case report Knee	20 patients: 9 males, 11 females, mean age: 59.6	18 mo	VAS, WOMAC	Pain relieved, scores improved
Hudetz *et al*[53] (2019)	SVF	Prospective, non-randomized and single center study Knee	20 patients: 15 males, 5 females	12 mo	VAS, WOMAC, KOOS	All scores improved, pain and symptoms relieved for up to a year
Berman *et al*[54] (2019) NCT10953523	SVF	Case report Knee	2586 patients	2-5 yr	Questionnaire (visual acuity pain scores, sustained improvement in function)	Over 80% of patients' pain relieved, joint function improved and maintain 1 yr; outcomes between male and female or between SVF alone and SVF + PRP showed no difference
Michalek *et al* [55] (2019)	SVF	Multicenter case-control study Knee and hip	29 patients: 9 males, 20 females, mean age: 83.3	36 mo	Modified KOOS/HOOS	Apart from 3 elderly patients died from aging, other patients' pain and weekly dosage of analgesics were reduced, KOOS/HOOS improved
Yokota *et al*[56] (2019)	ADSC *vs* SVF	Retrospective cohort study Knee/128 OA	ADSC: 42 patients; SVF: 38 patients	6 mo	VAS, KOOS	No major complications occurred, knee joint effusion was more likely to occur in SVF group than ADSC group (SVF 8%, ADSC 2%); VAS and KOOS improved in both groups
Mayoly *et al*[57] (2019)	SVF +PRP	Case report	3 patients: 1 male, 2 females, mean age: 62	12 mo	VAS, PRWE, DASH	All scores improved

NCT03164122		Wrist				
Hong *et al*[58] (2019)	SVF *vs* HA	Double-blind randomized self-controlled trial Knee	16 patients (with bilateral symptomatic knee OA) one side: SVF, the other side: HA. 3 males, 13 females, mean age: 52	12 mo	VAS, WOMAC, ROM, MRI (MOCART, WORMS)	Significant improvement of VAS, WOMAC and ROM in SVF group, but no improvement in HA group; SVF group was superior to HA group in cartilage repair showed by MOCART and WORMS
Tran *et al*[59] (2019)	SVF + AM *vs* AM	Single-center, non-randomized, placebo-controlled study Knee	33 patients, placebo group (AM): 3 males, 12 females, mean age: 58.2; SVF group (SVF + AM): 5 males, 13 females, mean age: 59	24 mo	VAS, WOMAC, Lysholm, OS	VAS, WOMAC of SVF group reduced significantly compared with placebo group, and maintained 24 mo; Lysholm, OS of SVF group improved
Garza *et al*[60] (2020) NCT02726945	SVF	Double-blinded prospective randomized controlled study Knee	39 patients (randomly assigned to high-, low-dose SVF, or placebo at 1:1:1)	12 mo	WOMAC, MRI	Symptoms and pain relieved by SVF treatment for up to at least 12 mo; changes in WOMAC scores reached statistical significance in the high- and low-dose groups compared to the placebo group; the improvements were dose dependent
Lapuente *et al* [61] (2020)	SVF	Retrospective study Knee/100 OA	50 patients: 28 males, 22 females, age: 50-89	12 mo	Lequesne, WOMAC, VAS	All scores improved
Tsubosaka *et al* [62] (2020)	SVF	Case report Knee	57 patients: 41 males, 16 females, mean age: 69.4	13.7 mo	ROM, WOMAC, VAS, KOOS, MRI	WOMAC, VAS and KOOS improved, while no significant difference in hip-knee-ankle angle, T2 mapping values of lateral femur and tibia improved significantly

HA: Hyaluronic acid; FRI: Functional rating index; MRI: Magnetic resonance imaging; VAS: Visual analogue score; ROM: Range of motion; WOMAC: Western Ontario and McMaster Universities Osteoarthritis Index; TUG: Timed up-and-go; JKOM: Japanese knee osteoarthritis measure; AM: Arthroscopic microfracture; OS: Outerbridge classification system; KOOS/HOOS: Knee/hip osteoarthritis outcome score; IKDC: International knee documentation committee-subjective; PRWE: Patient-rated wrist evaluation scores; DASH: Disabilities of the arm and shoulder; MOCART: Magnetic resonance observation of cartilage repair tissue score; BME: Bone marrow edema lesions; WORMS: Whole-organ magnetic resonance imaging score.



DOI: 10.4252/wjsc.v14.i10.744 **Copyright** ©The Author(s) 2022.

Figure 1 Procedures for isolating stromal vascular fraction to treat osteoarthritis. SVF: Stromal vascular fraction.

bone morphogenetic proteins (BMP-2, BMP-4, and BMP-7), insulin-like growth factor 1 (IGF-1), and fibroblast growth factor-2 (FGF-2). As a result, ADSCs may promote cartilage formation, induce cell proliferation, differentiation, and migration and ultimately promote cartilage injury repair[65].

Furthermore, ADSCs may secrete NO, TNF-α, IL, and other cytokines, which play a role in regulating the body's immunity and anti-inflammatory response[66]. At the same time, ADSCs can inhibit chondrocyte apoptosis by expressing antiapoptotic proteins, thereby slowing the onset of OA[67].

Many researchers have used PRP in combination with stem cells to treat OA and have achieved satisfactory therapeutic effects. This is because PRP can release a variety of growth factors, such as platelet-derived growth factor, TGF, FGF, and various ILs. These growth factors can not only regulate the body's immune response but can also promote and enhance the repair function of stem cells. Therefore, PRP can stimulate the proliferation and differentiation of chondrocytes, regulate the synthesis of endogenous hyaluronic acid, and help to repair cartilage tissue damage[68].

SVF is a multicellular component that, in addition to ADSCs, also contains progenitor cells, pericytes, endothelial cells, fibroblasts, and various immune cells. These cells also aid in the promotion of cartilage repair by forming the microenvironment, secreting cytokines, and regulating immunity[56].

DISCUSSION

Unfortunately, no complete cure exists for OA, and once a cartilage lesion occurs, it will gradually degenerate. As a result, the early diagnosis and treatment of OA are critical. Considering that osteoarthritis is a whole joint disease, OA should be treated with combined therapy. Through intraarticular injection therapy, treatment agents can directly reach the damaged site, which can not only allow drugs and especially stem cells to avoid being cleared by the body but also reduce the potential systemic effects of drugs[69]. Especially for traumatic arthritis, cartilage adipose stem cells provide a new avenue for the treatment of this type of arthritis through their powerful differentiation ability and paracrine and anti-inflammatory effects. Therefore, intraarticular injection therapy should be added to the combined treatment of OA. Increasing evidence shows that intraarticular injection of SVF is an effective treatment option for repairing articular cartilage damage, but there is a lack of clinical outcome data for long-term follow-up. Studies have indicated that the functional effect of cell therapy depends more on the quality of cytokines, chemokines and growth factors released by stem cells than on the number of stem cells. The stem cell activity of adipose-derived SVF was three times higher than that of bone marrow mesenchymal stem cells (BM-MSCs). At the same time, SVF does not require to be cultured *in vitro*, and its extraction and processing are also easier[70]. Therefore, AD-SVF has more advantages than BM-MSCs in the performance and ethical review of the treatment of osteoarthritis. Furthermore, the use of SVF to treat OA has certain individual differences, such as differences in extraction methods and equipment, which result in a variation in the number and quality of extracted cells. Simultaneously, the amount of fat acquired and the final cell yield of different patients are difficult to reconcile. The number of SVF cells used in the final treatment of OA varies by up to 10-fold in different studies. As a result, more detailed and comprehensive extraction standards and treatment guidelines for the use of SVF for OA treatment are needed.

The fact that SVF has a high safety profile in the treatment of OA is encouraging. Some researchers have used SVF in conjunction with other measures (such as the use of HA, microfracture, and PRP), and the results have been promising. As a result, in the future, a combination of multiple treatments, such as SVF combined with weight loss, exercise, and acupuncture to treat OA, may be considered, providing new treatment options for this disease. All of the patients who received SVF treatment had no serious adverse reactions, such as infection, acute pain, or cancer. Although patients occasionally experience minor reactions, such as joint effusion, swelling, pain congestion, and itching at the liposuction site, they can recover without intervention or improve with simple treatment. However, most clinical studies have certain limitations, such as a small number of clinical patients, a short follow-up period, and a lack of randomized controlled trials. As a result, in future studies, a more comprehensive and appropriate study design is needed. Recently, it has been concluded that clodronate can reduce pain and improve joint mobility by intraarticular injection for OA treatment. Combined with HA, clodronate can also relieve pain and reduce bone marrow lesions in early OA. In the future, it may be used in conjunction with SVF for better results[71].

CONCLUSION

In conclusion, SVF is an effective treatment for repairing articular cartilage damage, especially for relieving pain and other symptoms and improving joint function in OA patients. At the same time, clinical treatment with SVF is very safe. Relevant mechanistic studies revealed the beneficial role of SVF and its paracrine molecules in the treatment of osteoarthritis, which can mediate intercellular communication and interact with the cellular microenvironment and a variety of cell types, thus triggering appropriate cellular responses, inhibiting inflammation, promoting cartilage repair and regeneration and restoring joint homeostasis to reduce pain. However, various factors can change the characteristics of SVF and its secretion, such as individual differences in donors and different preparation standards, which may limit its therapeutic effect. Therefore, a further in-depth research is

still needed to make stem cells a routine clinical treatment for diseases such as osteoarthritis.

FOOTNOTES

Author contributions: Tang Q and Zhao XS contributed data collection and manuscript writing; Guo A, Cui RT, Song HL, Qi ZY, Pan Y, Yang Y contributed data analysis; Zhang FF, Jin L contributed study design and supervision; all authors have read and approve the final manuscript.

Supported by National Natural Science Foundation of China, No. 82070801, No. 82100858, No. 82073227; China Postdoctoral Science Foundation, No. 2020M671661; Jiangsu Province Science Foundation for Youths, No. BK20200569; and Jiangsu Province Research Founding for Postdoctoral, No. 1412000016.

Conflict-of-interest statement: All the authors report no relevant conflicts of interest for this article.

Country/Territory of origin: China

ORCID number: Liang Jin 0000-0002-1954-4345.

S-Editor: Gong ZM
L-Editor: A
P-Editor: Gong ZM

REFERENCES

1 **Lambova SN**, Müller-Ladner U. Osteoarthritis - Current Insights in Pathogenesis, Diagnosis and Treatment. *Curr Rheumatol Rev* 2018; **14**: 91-97 [PMID: 30003854 DOI: 10.2174/1573397114021807061447571]
2 **Georgiev T**, Angelov AK. Modifiable risk factors in knee osteoarthritis: treatment implications. *Rheumatol Int* 2019; **39**: 1145-1157 [PMID: 30911813 DOI: 10.1007/s00296-019-04290-z]
3 **Glyn-Jones S**, Palmer AJ, Agricola R, Price AJ, Vincent TL, Weinans H, Carr AJ. Osteoarthritis. *Lancet* 2015; **386**: 376-387 [PMID: 25748615 DOI: 10.1016/S0140-6736(14)60802-3]
4 **Safiri S**, Kolahi AA, Smith E, Hill C, Bettampadi D, Mansournia MA, Hoy D, Ashrafi-Asgarabad A, Sepidarkish M, Almasi-Hashiani A, Collins G, Kaufman J, Qorbani M, Moradi-Lakeh M, Woolf AD, Guillemin F, March L, Cross M. Global, regional and national burden of osteoarthritis 1990-2017: a systematic analysis of the Global Burden of Disease Study 2017. *Ann Rheum Dis* 2020; **79**: 819-828 [PMID: 32398285 DOI: 10.1136/annrheumdis-2019-216515]
5 **Wang T**, He C. Pro-inflammatory cytokines: The link between obesity and osteoarthritis. *Cytokine Growth Factor Rev* 2018; **44**: 38-50 [PMID: 30340925 DOI: 10.1016/j.cytogfr.2018.10.002]
6 **Kulkarni K**, Karssiens T, Kumar V, Pandit H. Obesity and osteoarthritis. *Maturitas* 2016; **89**: 22-28 [PMID: 27180156 DOI: 10.1016/j.maturitas.2016.04.006]
7 **Warner SC**, Valdes AM. Genetic association studies in osteoarthritis: is it fairytale? *Curr Opin Rheumatol* 2017; **29**: 103-109 [PMID: 27755178 DOI: 10.1097/BOR.0000000000000352]
8 **Suter LG**, Smith SR, Katz JN, Englund M, Hunter DJ, Frobell R, Losina E. Projecting Lifetime Risk of Symptomatic Knee Osteoarthritis and Total Knee Replacement in Individuals Sustaining a Complete Anterior Cruciate Ligament Tear in Early Adulthood. *Arthritis Care Res (Hoboken)* 2017; **69**: 201-208 [PMID: 27214559 DOI: 10.1002/acr.22940]
9 **Vaishya R**, Vijay V, Lama P, Agarwal A. Does vitamin D deficiency influence the incidence and progression of knee osteoarthritis? *J Clin Orthop Trauma* 2019; **10**: 9-15 [PMID: 30705525 DOI: 10.1016/j.jcot.2018.05.012]
10 **Alentorn-Geli E**, Samuelsson K, Musahl V, Green CL, Bhandari M, Karlsson J. The Association of Recreational and Competitive Running With Hip and Knee Osteoarthritis: A Systematic Review and Meta-analysis. *J Orthop Sports Phys Ther* 2017; **47**: 373-390 [PMID: 28504066 DOI: 10.2519/jospt.2017.7137]
11 **Nevitt MC**, Zhang Y, Javaid MK, Neogi T, Curtis JR, Niu J, McCulloch CE, Segal NA, Felson DT. High systemic bone mineral density increases the risk of incident knee OA and joint space narrowing, but not radiographic progression of existing knee OA: the MOST study. *Ann Rheum Dis* 2010; **69**: 163-168 [PMID: 19147619 DOI: 10.1136/ard.2008.099531]
12 **Marmor L**. Lateral compartment arthroplasty of the knee. *Clin Orthop Relat Res* 1984; 115-121 [PMID: 6723132]
13 **Sokolove J**, Lepus CM. Role of inflammation in the pathogenesis of osteoarthritis: latest findings and interpretations. *Ther Adv Musculoskelet Dis* 2013; **5**: 77-94 [PMID: 23641259 DOI: 10.1177/1759720X12467868]
14 **Rahmati M**, Nalesso G, Mobasheri A, Mozafari M. Aging and osteoarthritis: Central role of the extracellular matrix. *Ageing Res Rev* 2017; **40**: 20-30 [PMID: 28774716 DOI: 10.1016/j.arr.2017.07.004]
15 **Wojdasiewicz P**, Poniatowski ŁA, Szukiewicz D. The role of inflammatory and anti-inflammatory cytokines in the pathogenesis of osteoarthritis. *Mediators Inflamm* 2014; **2014**: 561459 [PMID: 24876674 DOI: 10.1155/2014/561459]

16 **Li G**, Yin J, Gao J, Cheng TS, Pavlos NJ, Zhang C, Zheng MH. Subchondral bone in osteoarthritis: insight into risk factors and microstructural changes. *Arthritis Res Ther* 2013; **15**: 223 [PMID: 24321104 DOI: 10.1186/ar4405]

17 **Holzer LA**, Kraiger M, Talakic E, Fritz GA, Avian A, Hofmeister A, Leithner A, Holzer G. Microstructural analysis of subchondral bone in knee osteoarthritis. *Osteoporos Int* 2020; **31**: 2037-2045 [PMID: 32472294 DOI: 10.1007/s00198-020-05461-6]

18 **Udomsinprasert W**, Jinawath A, Teerawattanapong N, Honsawek S. Interleukin-34 overexpression mediated through tumor necrosis factor-alpha reflects severity of synovitis in knee osteoarthritis. *Sci Rep* 2020; **10**: 7987 [PMID: 32409720 DOI: 10.1038/s41598-020-64932-2]

19 **Roemer FW**, Demehri S, Omoumi P, Link TM, Kijowski R, Saarakkala S, Crema MD, Guermazi A. State of the Art: Imaging of Osteoarthritis-Revisited 2020. *Radiology* 2020; **296**: 5-21 [PMID: 32427556 DOI: 10.1148/radiol.2020192498]

20 **Khatri C**, Dickenson E, Ahmed I, Bretherton C, Ranaboldo T, Shaw C, Quarcoopome J, Plastow R, Downham C, Rasidovic D, Plant C, Barlow T. ARthroscopy in Knee OsteoArthritis (ARK-OA): a multicentre study assessing compliance to national guidelines. *Eur J Orthop Surg Traumatol* 2021; **31**: 1443-1449 [PMID: 33611640 DOI: 10.1007/s00590-021-02905-5]

21 **Demehri S**, Guermazi A, Kwoh CK. Diagnosis and Longitudinal Assessment of Osteoarthritis: Review of Available Imaging Techniques. *Rheum Dis Clin North Am* 2016; **42**: 607-620 [PMID: 27742017 DOI: 10.1016/j.rdc.2016.07.004]

22 **Hayashi D**, Roemer FW, Guermazi A. Imaging for osteoarthritis. *Ann Phys Rehabil Med* 2016; **59**: 161-169 [PMID: 26797169 DOI: 10.1016/j.rehab.2015.12.003]

23 **Roemer FW**, Kwoh CK, Hayashi D, Felson DT, Guermazi A. The role of radiography and MRI for eligibility assessment in DMOAD trials of knee OA. *Nat Rev Rheumatol* 2018; **14**: 372-380 [PMID: 29752462 DOI: 10.1038/s41584-018-0010-z]

24 **Wakefield RJ**, Balint PV, Szkudlarek M, Filippucci E, Backhaus M, D'Agostino MA, Sanchez EN, Iagnocco A, Schmidt WA, Bruyn GA, Kane D, O'Connor PJ, Manger B, Joshua F, Koski J, Grassi W, Lassere MN, Swen N, Kainberger F, Klauser A, Ostergaard M, Brown AK, Machold KP, Conaghan PG; OMERACT 7 Special Interest Group. Musculoskeletal ultrasound including definitions for ultrasonographic pathology. *J Rheumatol* 2005; **32**: 2485-2487 [PMID: 16331793]

25 **Katz JN**, Brownlee SA, Jones MH. The role of arthroscopy in the management of knee osteoarthritis. *Best Pract Res Clin Rheumatol* 2014; **28**: 143-156 [PMID: 24792949 DOI: 10.1016/j.berh.2014.01.008]

26 **Saberi Hosnijeh F**, Bierma-Zeinstra SM, Bay-Jensen AC. Osteoarthritis year in review 2018: biomarkers (biochemical markers). *Osteoarthritis Cartilage* 2019; **27**: 412-423 [PMID: 30552966 DOI: 10.1016/j.joca.2018.12.002]

27 **Lotz M**, Martel-Pelletier J, Christiansen C, Brandi ML, Bruyère O, Chapurlat R, Collette J, Cooper C, Giacovelli G, Kanis JA, Karsdal MA, Kraus V, Lems WF, Meulenbelt I, Pelletier JP, Raynauld JP, Reiter-Niesert S, Rizzoli R, Sandell LJ, Van Spil WE, Reginster JY. Republished: Value of biomarkers in osteoarthritis: current status and perspectives. *Postgrad Med J* 2014; **90**: 171-178 [PMID: 24534711 DOI: 10.1136/postgradmedj-2013-203726rep]

28 **Bannuru RR**, Osani MC, Vaysbrot EE, Arden NK, Bennell K, Bierma-Zeinstra SMA, Kraus VB, Lohmander LS, Abbott JH, Bhandari M, Blanco FJ, Espinosa R, Haugen IK, Lin J, Mandl LA, Moilanen E, Nakamura N, Snyder-Mackler L, Trojian T, Underwood M, McAlindon TE. OARSI guidelines for the non-surgical management of knee, hip, and polyarticular osteoarthritis. *Osteoarthritis Cartilage* 2019; **27**: 1578-1589 [PMID: 31278997 DOI: 10.1016/j.joca.2019.06.011]

29 **Migliore A**, Paoletta M, Moretti A, Liguori S, Iolascon G. The perspectives of intra-articular therapy in the management of osteoarthritis. *Expert Opin Drug Deliv* 2020; **17**: 1213-1226 [PMID: 32543240 DOI: 10.1080/17425247.2020.1783234]

30 **Belsey J**, Yasen SK, Jobson S, Faulkner J, Wilson AJ. Return to Physical Activity After High Tibial Osteotomy or Unicompartmental Knee Arthroplasty: A Systematic Review and Pooling Data Analysis. *Am J Sports Med* 2021; **49**: 1372-1380 [PMID: 32960075 DOI: 10.1177/0363546520948861]

31 **Klag KA**, Horton WA. Advances in treatment of achondroplasia and osteoarthritis. *Hum Mol Genet* 2016; **25**: R2-R8 [PMID: 26443596 DOI: 10.1093/hmg/ddv419]

32 **Shah FS**, Wu X, Dietrich M, Rood J, Gimble JM. A non-enzymatic method for isolating human adipose tissue-derived stromal stem cells. *Cytotherapy* 2013; **15**: 979-985 [PMID: 23725689 DOI: 10.1016/j.jcyt.2013.04.001]

33 **Markarian CF**, Frey GZ, Silveira MD, Chem EM, Milani AR, Ely PB, Horn AP, Nardi NB, Camassola M. Isolation of adipose-derived stem cells: a comparison among different methods. *Biotechnol Lett* 2014; **36**: 693-702 [PMID: 24322777 DOI: 10.1007/s10529-013-1425-x]

34 **Busser H**, De Bruyn C, Urbain F, Najar M, Pieters K, Raicevic G, Meuleman N, Bron D, Lagneaux L. Isolation of adipose-derived stromal cells without enzymatic treatment: expansion, phenotypical, and functional characterization. *Stem Cells Dev* 2014; **23**: 2390-2400 [PMID: 24805167 DOI: 10.1089/scd.2014.0071]

35 **Zhao X**, Guo J, Zhang F, Zhang J, Liu D, Hu W, Yin H, Jin L. Therapeutic application of adipose-derived stromal vascular fraction in diabetic foot. *Stem Cell Res Ther* 2020; **11**: 394 [PMID: 32928305 DOI: 10.1186/s13287-020-01825-1]

36 **Bourin P**, Bunnell BA, Casteilla L, Dominici M, Katz AJ, March KL, Redl H, Rubin JP, Yoshimura K, Gimble JM. Stromal cells from the adipose tissue-derived stromal vascular fraction and culture expanded adipose tissue-derived stromal/stem cells: a joint statement of the International Federation for Adipose Therapeutics and Science (IFATS) and the International Society for Cellular Therapy (ISCT). *Cytotherapy* 2013; **15**: 641-648 [PMID: 23570660 DOI: 10.1016/j.jcyt.2013.02.006]

37 **Baer PC**, Geiger H. Adipose-derived mesenchymal stromal/stem cells: tissue localization, characterization, and heterogeneity. *Stem Cells Int* 2012; **2012**: 812693 [PMID: 22577397 DOI: 10.1155/2012/812693]

38 **Miana VV**, González EAP. Adipose tissue stem cells in regenerative medicine. *Ecancermedicalscience* 2018; **12**: 822 [PMID: 29662535 DOI: 10.3332/ecancer.2018.822]

39 **Aust L**, Devlin B, Foster SJ, Halvorsen YD, Hicok K, du Laney T, Sen A, Willingmyre GD, Gimble JM. Yield of human adipose-derived adult stem cells from liposuction aspirates. *Cytotherapy* 2004; **6**: 7-14 [PMID: 14985162 DOI: 10.1080/14653240310004539]

40 **Strioga M**, Viswanathan S, Darinskas A, Slaby O, Michalek J. Same or not the same? *Stem Cells Dev* 2012; **21**: 2724-2752 [PMID: 22468918 DOI: 10.1089/scd.2011.0722]

41 **Pak J**, Lee JH, Park KS, Jeong BC, Lee SH. Regeneration of Cartilage in Human Knee Osteoarthritis with Autologous Adipose Tissue-Derived Stem Cells and Autologous Extracellular Matrix. *Biores Open Access* 2016; **5**: 192-200 [PMID: 27588219 DOI: 10.1089/biores.2016.0024]

42 **Pak J**, Lee JH, Pak NJ, Park KS, Jeon JH, Jeong BC, Lee SH. Clinical Protocol of Producing Adipose Tissue-Derived Stromal Vascular Fraction for Potential Cartilage Regeneration. *J Vis Exp* 2018 [PMID: 30320755 DOI: 10.3791/58363]

43 **Fodor PB**, Paulseth SG. Adipose Derived Stromal Cell (ADSC) Injections for Pain Management of Osteoarthritis in the Human Knee Joint. *Aesthet Surg J* 2016; **36**: 229-236 [PMID: 26238455 DOI: 10.1093/asj/sjv135]

44 **Yokota N**, Yamakawa M, Shirata T, Kimura T, Kaneshima H. Clinical results following intra-articular injection of adipose-derived stromal vascular fraction cells in patients with osteoarthritis of the knee. *Regen Ther* 2017; **6**: 108-112 [PMID: 30271845 DOI: 10.1016/j.reth.2017.04.002]

45 **Nguyen PD**, Tran TD, Nguyen HT, Vu HT, Le PT, Phan NL, Vu NB, Phan NK, Van Pham P. Comparative Clinical Observation of Arthroscopic Microfracture in the Presence and Absence of a Stromal Vascular Fraction Injection for Osteoarthritis. *Stem Cells Transl Med* 2017; **6**: 187-195 [PMID: 28170179 DOI: 10.5966/sctm.2016-0023]

46 **Michalek J**, Moster R, Lukac L, Proefrock K, Petrasovic M, Rybar J, Chaloupka A, Darinskas A, Michalek J, Kristek J, Travnik J, Jabandziev P, Cibulka M, Skopalik J, Kristkova Z, Dudasova Z. Stromal Vascular Fraction Cells of Adipose and Connective Tissue in People with Osteoarthritis: A Case Control Prospective Multi-Centric Non-Randomized Study. *Global Surg* 2017; **3**: 1-9 [DOI: 10.15761/GOS.1000163]

47 **Tantuway V**, Sharma AK, Mehta MH, Sharma R, Mantry P, Mehto P. Use of Autologous Adipose-derived Stromal Vascular Fraction Grafting in Treatment of Knee Osteoarthritis: A Safety and Efficacy Study. *J Med Res Prac* 2017; **6**: 119-127

48 **Russo A**, Condello V, Madonna V, Guerriero M, Zorzi C. Autologous and micro-fragmented adipose tissue for the treatment of diffuse degenerative knee osteoarthritis. *J Exp Orthop* 2017; **4**: 33 [PMID: 28975547 DOI: 10.1186/s40634-017-0108-2]

49 **Russo A**, Screpis D, Di Donato SL, Bonetti S, Piovan G, Zorzi C. Autologous micro-fragmented adipose tissue for the treatment of diffuse degenerative knee osteoarthritis: an update at 3 year follow-up. *J Exp Orthop* 2018; **5**: 52 [PMID: 30569417 DOI: 10.1186/s40634-018-0169-x]

50 **Bright B**, Bright R, Bright P, Limaye A. Ankylosing spondylitis, chronic fatigue and depression improved after stromal vascular fraction treatment for osteoarthritis: a case report. *J Med Case Rep* 2018; **12**: 238 [PMID: 30153860 DOI: 10.1186/s13256-018-1776-y]

51 **Barfod KW**, Blønd L. Treatment of osteoarthritis with autologous and microfragmented adipose tissue. *Dan Med J* 2019; **66** [PMID: 31571571]

52 **Roato I**, Belisario DC, Compagno M, Lena A, Bistolfi A, Maccari L, Mussano F, Genova T, Godio L, Perale G, Formica M, Cambieri I, Castagnoli C, Robba T, Felli L, Ferracini R. Concentrated adipose tissue infusion for the treatment of knee osteoarthritis: clinical and histological observations. *Int Orthop* 2019; **43**: 15-23 [PMID: 30311059 DOI: 10.1007/s00264-018-4192-4]

53 **Hudetz D**, Borić I, Rod E, Jeleč Ž, Kunovac B, Polašek O, Vrdoljak T, Plečko M, Skelin A, Polančec D, Zenić L, Primorac D. Early results of intra-articular micro-fragmented lipoaspirate treatment in patients with late stages knee osteoarthritis: a prospective study. *Croat Med J* 2019; **60**: 227-236 [PMID: 31187950]

54 **Berman M**, Lander E, Grogan T, O'Brien W, Braslow J, Dowell S, Berman S. Prospective Study of Autologous Adipose Derived Stromal Vascular Fraction Containing Stem Cells for the Treatment of Knee Osteoarthritis. *In J Stem Cell Res Ther* 2019; **6**: 064 [DOI: 10.23937/2469-570X/1410064]

55 **Michalek J**, Vrablikova A, Darinskas A, Lukac L, Prucha J, Skopalik J, Travnik J, Cibulka M, Dudasova Z. Stromal vascular fraction cell therapy for osteoarthritis in elderly: Multicenter case-control study. *J Clin Orthop Trauma* 2019; **10**: 76-80 [PMID: 30705536 DOI: 10.1016/j.jcot.2018.11.010]

56 **Yokota N**, Hattori M, Ohtsuru T, Otsuji M, Lyman S, Shimomura K, Nakamura N. Comparative Clinical Outcomes After Intra-articular Injection With Adipose-Derived Cultured Stem Cells or Noncultured Stromal Vascular Fraction for the Treatment of Knee Osteoarthritis. *Am J Sports Med* 2019; **47**: 2577-2583 [PMID: 31373830 DOI: 10.1177/0363546519864359]

57 **Mayoly A**, Iniesta A, Curvale C, Kachouh N, Jaloux C, Eraud J, Vogtensperger M, Veran J, Grimaud F, Jouve E, Casanova D, Sabatier F, Legré R, Magalon J. Development of Autologous Platelet-Rich Plasma Mixed-Microfat as an Advanced Therapy Medicinal Product for Intra-Articular Injection of Radio-Carpal Osteoarthritis: From Validation Data to Preliminary Clinical Results. *Int J Mol Sci* 2019; **20** [PMID: 30841510 DOI: 10.3390/ijms20051111]

58 **Hong Z**, Chen J, Zhang S, Zhao C, Bi M, Chen X, Bi Q. Intra-articular injection of autologous adipose-derived stromal vascular fractions for knee osteoarthritis: a double-blind randomized self-controlled trial. *Int Orthop* 2019; **43**: 1123-1134 [PMID: 30109404 DOI: 10.1007/s00264-018-4099-0]

59 **Tran TDX**, Wu CM, Dubey NK, Deng YH, Su CW, Pham TT, Thi Le PB, Sestili P, Deng WP. Time- and Kellgren–Lawrence Grade-Dependent Changes in Intra-Articularly Transplanted Stromal Vascular Fraction in Osteoarthritic Patients. *Cells* 2019; **8** [PMID: 30987218 DOI: 10.3390/cells8040308]

60 **Garza JR**, Campbell RE, Tjoumakaris FP, Freedman KB, Miller LS, Santa Maria D, Tucker BS. Clinical Efficacy of Intra-articular Mesenchymal Stromal Cells for the Treatment of Knee Osteoarthritis: A Double-Blinded Prospective Randomized Controlled Clinical Trial. *Am J Sports Med* 2020; **48**: 588-598 [PMID: 32109160 DOI: 10.1177/0363546519899923]

61 **Lapuente JP**, Dos-Anjos S, Blázquez-Martínez A. Intra-articular infiltration of adipose-derived stromal vascular fraction cells slows the clinical progression of moderate-severe knee osteoarthritis: hypothesis on the regulatory role of intra-articular adipose tissue. *J Orthop Surg Res* 2020; **15**: 137 [PMID: 32272946 DOI: 10.1186/s13018-020-01664-z]

62 **Tsubosaka M**, Matsumoto T, Sobajima S, Matsushita T, Iwaguro H, Kuroda R. The influence of adipose-derived stromal vascular fraction cells on the treatment of knee osteoarthritis. *BMC Musculoskelet Disord* 2020; **21**: 207 [PMID: 32252731 DOI: 10.1186/s12891-020-03231-3]

63 **Sohni A**, Verfaillie CM. Mesenchymal stem cells migration homing and tracking. *Stem Cells Int* 2013; **2013**: 130763 [PMID: 24194766 DOI: 10.1155/2013/130763]

64 **Docheva D**, Popov C, Mutschler W, Schieker M. Human mesenchymal stem cells in contact with their environment: surface characteristics and the integrin system. *J Cell Mol Med* 2007; **11**: 21-38 [PMID: 17367499 DOI: 10.1111/j.1582-4934.2007.00001.x]

65 **Richardson SM**, Kalamegam G, Pushparaj PN, Matta C, Memic A, Khademhosseini A, Mobasheri R, Poletti FL, Hoyland JA, Mobasheri A. Mesenchymal stem cells in regenerative medicine: Focus on articular cartilage and intervertebral disc regeneration. *Methods* 2016; **99**: 69-80 [PMID: 26384579 DOI: 10.1016/j.ymeth.2015.09.015]

66 **Yi T**, Song SU. Immunomodulatory properties of mesenchymal stem cells and their therapeutic applications. *Arch Pharm Res* 2012; **35**: 213-221 [PMID: 22370776 DOI: 10.1007/s12272-012-0202-z]

67 **Shang J**, Liu H, Li J, Zhou Y. Roles of hypoxia during the chondrogenic differentiation of mesenchymal stem cells. *Curr Stem Cell Res Ther* 2014; **9**: 141-147 [PMID: 24372326 DOI: 10.2174/1574888x09666131230142459]

68 **Nöth U**, Steinert AF, Tuan RS. Technology insight: adult mesenchymal stem cells for osteoarthritis therapy. *Nat Clin Pract Rheumatol* 2008; **4**: 371-380 [PMID: 18477997 DOI: 10.1038/ncprheum0816]

69 **Georgiev T**. Multimodal approach to intraarticular drug delivery in knee osteoarthritis. *Rheumatol Int* 2020; **40**: 1763-1769 [PMID: 32803403 DOI: 10.1007/s00296-020-04681-7]

70 **Jeyaraman M**, Muthu S, Ganie PA. Does the Source of Mesenchymal Stem Cell Have an Effect in the Management of Osteoarthritis of the Knee? *Cartilage* 2021; **13**: AAAA1532-1547 [PMID: 32840122 DOI: 10.1177/1947603520951623]

71 **Moretti A**, Paoletta M, Liguori S, Ilardi W, Snichelotto F, Toro G, Gimigliano F, Iolascon G. The Rationale for the Intra-Articular Administration of Clodronate in Osteoarthritis. *Int J Mol Sci* 2021; **22** [PMID: 33799992 DOI: 10.3390/ijms22052693]

World Journal of Stem Cells

Submit a Manuscript: https://www.f6publishing.com

World J Stem Cells 2022 October 26; 14(10): 756-776

DOI: 10.4252/wjsc.v14.i10.756

ISSN 1948-0210 (online)

ORIGINAL ARTICLE

Basic Study

Maternal inappropriate calcium intake aggravates dietary-induced obesity in male offspring by affecting the differentiation potential of mesenchymal stem cells

Ping Li, Yang Wang, Pei Li, Yuan-Lin Liu, Wei-Jiang Liu, Xiao-Yu Chen, Tian-Tian Tang, Ke-Min Qi, Yi Zhang

Specialty type: Cell and tissue engineering

Provenance and peer review: Unsolicited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's scientific quality classification

Grade A (Excellent): A
Grade B (Very good): B
Grade C (Good): C
Grade D (Fair): 0
Grade E (Poor): 0

P-Reviewer: Li Y, China; Prasetyo EP, Indonesia; Wei Z, United States

Received: May 5, 2022
Peer-review started: May 5, 2022
First decision: June 11, 2022
Revised: June 24, 2022
Accepted: August 7, 2022
Article in press: August 7, 2022
Published online: October 26, 2022

Ping Li, Xiao-Yu Chen, Tian-Tian Tang, Ke-Min Qi, Laboratory of Nutrition and Development, Key Laboratory of Major Diseases in Children's Ministry of Education, Beijing Pediatric Research Institute, Beijing Children's Hospital, Capital Medical University, National Center for Children's Health, Beijing 100045, China

Yang Wang, Yuan-Lin Liu, Wei-Jiang Liu, Yi Zhang, Department of Experimental Hematology and Biochemistry, Beijing Institute of Radiation Medicine, Beijing 100085, China

Pei Li, Department of Pediatrics, General Hospital of Tianjin Medical University, Tianjin Medical University, Tianjin 300070, China

Corresponding author: Yi Zhang, MD, PhD, Professor, Department of Experimental Hematology and Biochemistry, Beijing Institute of Radiation Medicine, No. 27 Tai-ping Road, Beijing 100085, China. zhangyi612@hotmail.com

Abstract

BACKGROUND

The effects of inappropriate dietary calcium intake in early life on later obesity have not been fully elucidated.

AIM

To raise the mechanism of maternal calcium intake on the multi-differentiation potential of mesenchymal stem cells among their male offspring.

METHODS

Four-week-old female C57BL/6N mice were fed by deficient, low, normal and excessive calcium reproductive diets throughout pregnancy and lactation. Bone MSCs (BMSCs) were obtained from 7-day-old male offspring to measure the adipogenic differentiation potential by the Wnt/β-catenin signaling pathway. The other weaning male pups were fed a high-fat diet for 16 wk, along with normal-fat diet as the control. Then the serum was collected for the measurement of biochemical indicators. Meanwhile, the adipose tissues were excised to analyze the adipocyte sizes and inflammatory infiltration. And the target gene expressions on the adipogenic differentiation and Wnt/β-catenin signaling pathway in the adipose tissues and BMSCs were determined by real-time reverse transcription polymerase chain reaction.

RESULTS

Compared with the control group, maternal deficient, low and excessive calcium intake during pregnancy and lactation aggravated dietary-induced obesity, with larger adipocytes, more serious inflammatory infiltration and higher serum metabolism indicators by interfering with higher expressions of adipogenic differentiation (*PPARγ, C/EBPα, Fabp4, LPL, Adiponectin, Resistin* and/or *Leptin*) among their male offspring ($P < 0.05$). And there were significantly different expression of similar specific genes in the BMSCs to successfully polarize adipogenic differentiation and suppress osteogenic differentiation *in vivo* and *in vitro*, respectively ($P < 0.05$). Meanwhile, it was accompanied by more significant disorders on the expressions of Wnt/β-catenin signaling pathway both in BMSCs and adulthood adipose tissues among the offspring from maternal inappropriate dietary calcium intake groups.

CONCLUSION

Early-life abnormal dietary calcium intake might program the adipogenic differentiation potential of BMSCs from male offspring, with significant expressions on the Wnt/β-catenin signaling pathway to aggravate high-fat-diet-induced obesity in adulthood.

Key Words: Calcium; Obesity; Bone mesenchymal stem cells; Wnt/β-catenin signaling pathway; Adipogenic differentiation; Male offspring

Core tip: Maternal inappropriate dietary calcium intake could aggravate high-fat-diet-induced obesity among male offspring, with larger adipocytes and more serious inflammatory infiltration by interfering with the higher expressions of adipogenic genes, which was accompanied by significant expressions of specific genes on the adipogenic and osteogenic differentiation. It was worsened by the disorders of Wnt/β-catenin signaling pathway both in the BMSCs and adipose tissues. So the importance of this study was that the prevention of adulthood obesity could be moved forward to the appropriate calcium intake in the neonatal period, even the formation of maternal germ cells and fertilized egg.

Citation: Li P, Wang Y, Li P, Liu YL, Liu WJ, Chen XY, Tang TT, Qi KM, Zhang Y. Maternal inappropriate calcium intake aggravates dietary-induced obesity in male offspring by affecting the differentiation potential of mesenchymal stem cells. *World J Stem Cells* 2022; 14(10): 756-776
URL: https://www.wjgnet.com/1948-0210/full/v14/i10/756.htm
DOI: https://dx.doi.org/10.4252/wjsc.v14.i10.756

INTRODUCTION

Obesity has become a worldwide noncommunicable health crisis with rising prevalence in the past few decades due to excess calorie intake, fat accumulation, and adiposity[1,2]. It can cause severe metabolic disorders such as nonalcoholic steatohepatitis, type 2 diabetes, cardiovascular diseases, and cancer[1-3]. These above pathological complications are characterized by more hypertrophy and hyperplasia of adipocytes to cause dynamic expansion in the adipose tissues, in which hyperplasia is a complicated process including disruption of the commitment of mesenchymal stem cells (MSCs) to form preadipocytes, and terminal differentiation from preadipocytes to mature adipocytes[4-6]. And MSCs (CD29+, CD90+, Sca-1+, CD31-, CD34-, CD45− and CD49d−), as a group of cells with multi-lineage differentiated potential and self-renewal capacity, are the major original sources of mature adipocytes. , in which the key coordinated cascade of transcription factors were mainly included *PPARγ*, *C/EBPα*, *LPL* and *FABP4*, with the significant secretory molecules such as *Leptin*, *Adiponectin* and *Resistin*[7,8]. In this process, the mechanisms governing the adipogenic differentiation of MSCs can be regulated by the coordination of complex networks in many signaling pathways, such as JAK2/STAT3, SIRT1/SIRT2, ERK1/ERK2, TGF-β/BMP, Wnt/β-catenin and RHO-family GTPase[9,10], in which the activation of Wnt/β-catenin signaling can inhibit adipogenic differentiation and promote osteogenic differentiation through endogenous regulatory genes (*CTNNB1, Wnt1, Wnt10a, Wnt10b, Wnt5a, Gsk3β, Axin2* and *TGF7L2*)[11,12]. It has been demonstrated that the differentiation potential of MSCs mainly occurs in early life, and the numbers and differentiation potential significantly decline with the age[12], so the nutritional status and exposure to adverse factors at this stage, especially pregnancy and lactation, are important for the differentiation potential of MSCs to affect later metabolic disturbances in adulthood [13-16].

Calcium is an important functional nutrient on the regulation of energy balance and glucose uptake in the battle against obesity[17-19]. However, daily calcium intake is still lower than its recommended nutrient intake among pregnant women[20,21], so the imbalance of calcium intake in early life may have detrimental effects on later health. Both our high-fat-diet (HFD) induced obese mouse mode and epidemiological cohorts showed that both dietary insufficient and excessive calcium intake during pregnancy and lactation increased body weight gain by affecting the gut microbiota structure, and abnormal expression of lipolysis and liposynthesis among their male offspring [22-24]. However, the specific mechanisms by which maternal calcium intake modulates body weight and fat and glucose homeostasis of their infants are still not fully understood. Some research had found that Ca^{2+} formed in the culture medium had osteo-inductive properties to promote osteogenic differentiation of MSCs[25]. Previous studies also had demonstrated that neonatal calcium deficiency could reduce the osteogenic priming of MSCs by enlarging the subpopulation with adipogenic potential in piglets and mice *in vivo* [26]. Furthermore, it is competing and reciprocal on the balance of adipogenic and osteogenic differentiation of MSCs[27,28]. However, whether maternal inappropriate dietary calcium intake can increase the adipogenic differentiation potential of MSCs among their male offspring is still unclear.

Thus, this study was designed to investigate whether abnormal dietary calcium intake during gestation and lactation affected the multi-differentiation potential of bone MSCs (BMSCs) to aggravate the development of adulthood obesity among their male offspring and explore the possible signaling pathways. This deeper understanding of early-life calcium intake could play a significant role on preventing later obesity.

MATERIALS AND METHODS

Animal procedures

Sixty 4-week-old C57BL/6N female mice were obtained from Beijing Vital River Laboratory Animal Technology (License SCXK-Beijing) and housed at the Animal Center in the Academy of Military Medical Sciences under a 12-h light/dark cycle (lights-on 08:00 h) with adequate food and water intake at 22°C and 50% humidity. All mice were randomly divided into four groups (n = 15/group) and fed with the deficient (DC, 0.05%), low (LC, 0.25%), normal (NC, 0.70%) and high-calcium (HC, 1.20%) reproductive diets respectively for 6 wk. Five mice in each group (n = 5/group) were killed to determine the maternal contents of calcium and other metabolic indicators in the serum before mating. Then the remaining mice (n = 10/group) were mated with 10-week-old C57BL/6N male mice from Beijing Vital River Laboratory Animal Technology (2:1/cage), and continued on their own diets throughout gestation and lactation. According to the previous studies[21-23], the male offspring were used to study the development of obesity after the different calcium interventions during pregnancy and lactation. The 7-day-old male offspring (n = 9/group from more than three cages) in each group were killed to obtain BMSCs. While at age 21 d, the male offspring in the DC, LC, NC and HC groups (n = 10/group) were respectively weaned onto the HFD (34.9% fat by weight, 60% kcal, No. H10060) for 16 wk (NC-HFD, DC-HFD, LC-HFD and HC-HFD groups); with the normal fat diet (4.3% fat by weight, 10% kcal, No. H10010) as the control (NC-C group). All above diets were prepared by Beijing HFK Bioscience Co. Ltd. (http://www.hfkbio.com/) (Table 1). Body weight, food intake and energy intake in the NC-C, NC-HFD, DC-HFD, LC-HFD and HC-HFD groups were recorded weekly. Their blood samples were collected through the eye-drop, then they were anesthetized by the carbon dioxide inhalation. Immediately, their adipose tissues including the epididymal white adipose tissue (eWAT), inguinal white adipose tissue (iWAT), kidney adipose tissue (KAT) and brown adipose tissue (BAT) were freely dissected from the surrounding tissues, in which some were fixed in 10% phosphate-buffered formalin, some were stored in phosphate-buffered saline (PBS) to analyze the percentage of immune cells, and the remainder was frozen in liquid N_2. The serum samples were separated at 3000 r/min for 15 min after they were kept for 30 min at room temperature. All biological samples were stored in a –80°C refrigerator until use.

All animal studies were approved and conducted in accordance with the Beijing Academy of Military Medical Sciences Guide for the Care and Usage Committee of Laboratory Animals. The animal protocol was approved by the Ethics of Animal Experiments in the Academy of Military Medical Sciences in China (No. IACUC-DWZX-2019-704).

Measurement of the biochemical indicators

The concentrations of serum calcium, glucose, triglyceride (TG) and total cholesterol (TC) were respectively measured by the coloristic methods using the enzymatic assay kits (Maccura Biotechnology Co. Ltd., Sichuan, China). The male offspring mice in each group were orally gavaged with 20% glucose (weight/volume: 2.0g/kg) after a 10-h overnight fast and blood samples were collected from the tail vein at 15, 30, 60, 90 and 120 min to determine the glucose content (oral glucose tolerance test, OGTT) at 13 wk. The insulin tolerance test (ITT) was performed 1 wk after the OGTT, in which the blood samples were collected from the tail vein for the determination of blood glucose after 2 h fasting.

Table 1 Details of diet formulations in this study (g/kg diet)

Ingredients (g)	Reproductive diets				Feeding diets (0.70%)	
	Deficient calcium diet (0.05%)	Low calcium diet (0.25%)	Normal calcium diet (0.70%)	High calcium diet (1.20%)	Normal fat diet (H10010)	High fat diet (H10060)
Casein	200.00	200.00	200.00	200.00	189.58	258.45
Cystine	3.00	3.00	3.00	3.00	2.84	3.88
Cornstarch	396.30	391.30	380.00	367.50	298.59	161.53
Maltodextrin	132.00	132.00	132.00	132.00	33.18	88.91
Sucrose	100.00	100.00	100.00	100.00	331.77	---
Fibrin	50.00	50.00	50.00	50.00	47.40	64.61
Soybean oil	70.00	70.00	70.00	70.00	23.70	32.31
Lard oil	---	---	---	---	18.96	316.60
Mineral mixture without calcium (M1004)	35.00	35.00	35.00	35.00	---	---
Mineral mixture (M1002)	---	---	---	---	9.48	12.92
Calcium bicarbonate	---	---	---	---	12.32	16.80
Calcium carbonate ($CaCO_3$)	1.25	6.25	17.50	30.00	5.21	7.11
Potassium citrate. H_2O	---	---	---	---	15.64	21.32
Vitamin mixture (V1002)	10.00	10.00	10.00	10.00	9.48	12.92
Choline Bitartrate	2.50	2.50	2.50	2.50	1.90	2.58
Antioxidant (TBHQ)	0.014	0.014	0.014	0.014	0.047	0.065

Analysis of the immune cells in the adipose tissues

Stromal vascular fraction cells (SVFs) were extracted from the eWAT and iWAT in PBS. The infiltration and percentages of M1 macrophages ($CD45^+CD64^+CD11C^+$), M2 macrophages ($CD45^+CD64^+CD11C^-$) and adipose tissue dendritic cells (ATDCs, $CD45^+CD64^-CD11C^+$) were determined using the BD FACSCanto II Flow Cytometer (BD Biosciences, USA), and analyzed by FlowJo flow cytometry software (Treestar Inc., Ashland, OH, USA).

Histological analysis of adipose tissues

eWAT, iWAT, KAT and BAT in the NC-C, DC-HFD, LC-HFD, NC-HFD and HC-HFD groups were embedded in paraffin and cut into 6-μm sections, and stained with hematoxylin and eosin to measure the adipocyte size and inflammatory infiltration under a light microscope at 200× magnification, and analyzed by Image-pro Plus. All above histological experiments were performed by Servicebio (Beijing, China).

Gene expression related to adipogenic differentiation and Wnt/β-catenin signaling pathway in adipose tissues

Total RNA in eWAT, iWAT, KAT and BAT was extracted using TRIzol Reagent (Invitrogen, Carlsbad, CA, USA), and cDNA was reverse transcribed by Transcript® One-Step gDNA Removal and cDNA Synthesis SuperMix (TransGen Biotech, China).

The genes related to adipogenic differentiation (*PPARγ*, *C/EBPα*, *LPL*, *Fabp4*, *Adiponectin*, *Resistin* and *Leptin*) and Wnt/β-catenin signaling pathway (*CTNNB1*, *Wnt1*, *Wnt10a*, *Wnt10b*, *Wnt5a*, *Gsk3β*, *Axin2* and *TGF7L2*) were determined by real-time reverse transcription polymerase chain reaction (RT-PCR) (No. AQ101-03, TransStart® Green qPCR SuperMix, TransGen Biotech, China) (CFX-96; Bio-Rad, USA), and 36B4 was the invariant internal gene (Supplementary Table 1). Gene expression was normalized using the 2^{CT} method.

BMSC derivation and maintenance from male offspring

BMSCs from 7-day-old male offspring in the NC, DC, LC and HC groups were isolated and cultured as follows. The tibia and fibula from three pups with different mothers were isolated after washing with PBS to remove the residual muscle and blood under sterile conditions. They were shredded into small pieces of 2 mm^3 and digested in 0.1% type II collagenase (Gibco) at 37C for 40 min, and transferred into α

-minimal essential medium (α-MEM) (Gibco) containing 10% fetal bovine serum (FBS; Gibco), 100 U/mL penicillin, and 100 mg/mL streptomycin. The medium with FBS was changed every 3 d. When the adherent BMSCs reached 80%–90% confluence, they were collected in 0.25% trypsin (Gibco) and subcultured at a ratio of 1:3 for the further expansion and identification until the P3 generation, and used for subsequent experiments.

Detection of cell cycle and surface antibodies of BMSCs by flow cytometry

P3 generation BMSCs (1×10^6) from the DC, LC, NC and HC groups were phenotypically fixed, stained and characterized by the antibody permeabilization process. Mouse phycoerythrin-conjugated monoclonal antibodies Sca-1 (AB_2539218, MA5-17834), CD90 (AB_469640, 25-0900-82) and CD31 (AB_657735, 17-0311-82) (eBioscience, Waltham, MA, USA), and fluorescein-isothiocyanate-conjugated antibodies including CD29 (AB_2572449, 11-0291-82), CD34 (AB_465021, 11-0341-82) and CD45 (AB_465050, 11-0451-82) and CD49d (AB_465083, 11-0492-82) (eBioscience) were used to detect the purity of BMSCs. The Cell Cycle and Apoptosis Analysis kit (Beyotime) was obtained to measure the cell cycle of BMSCs. All above signals were recorded by flow cytometry with the FACScalibur system (Becton Dickinson) and analyzed using FlowJo software (Supplementary Figure 1 and Table 2).

Differentiation potential of BMSCs

To identify the adipogenic differentiation potential of BMSCs in the NC, DC, LC and HC groups, P3 BMSCs (8×10^4) were cultured with α-MEM containing 10% FBS and the related adipogenic inducer (10^3 mM dexamethasone, 0.5 mM isobutyl methylxanthine, 0.2 mM indomethacin, and 10 μg/mL insulin) (Sigma, Germany) for 7 d, in which the medium was changed every 3 d. Self-differentiated BMSCs without the above inducers (3×10^4) were as the controls. The induced and self-differentiated BMSCs were stained with Oil Red O (Sigma, Germany) and measured the gene expression related to adipogenic differentiation[29].

The osteogenic differentiation capacity of BMSCs was assessed by incubating the cells (3×10^4) with α-MEM containing 10% FBS and osteogenic inducer (10^7 mM dexamethasone, 0.5 mM ascorbic acid, and 10 mM β-glycerol phosphate) (Sigma, Germany) for 10 d, while the self-differentiated BMSCs without the above inducers (3×10^4) were as the controls. To demonstrate the osteogenic differentiation capacity of BMSCs, they were identified by immunocytochemical staining with alkaline phosphatase[29]. Expression of genes related to osteogenic differentiation were determined by RT-PCR.

Quantitative RT-PCR of BMSCs

Total RNA was extracted from the P3 BMSCs, adipogenic and osteogenic differentiated BMSCs and their related self-differentiated BMSCs using TRIzol Reagent (Invitrogen), and their cDNA samples were reverse transcribed by Transcript® One-Step gDNA Removal and cDNA Synthesis SuperMix (TransGen Biotech, China). The genes involved in adipogenic differentiation (*PPARγ*, *C/EBPα*, *LPL*, *Fabp4*, *Adiponectin*, *Resistin* and *Leptin*), osteogenic differentiation (*Runx2*, *ALP*, *COL1A1*, *Osteocalcin* and *Osteopontin*) and Wnt/β-catenin signaling pathway (*Wnt1*, *Wnt10a*, *Wnt10b*, *Wnt5a*, *CTNNB1*, *Gsk3β*, *Axin2* and *TGF7L2*) (Supplementary Table 1) were determined by the RT-PCR (No. AQ101-03, TransStart® Green qPCR SuperMix, TransGen Biotech, China), and 36B4 was the invariant internal control. The assays were performed in triplicate and normalized to the internal standard mRNA levels using the 2^{CT} method.

Statistical analysis

All statistical analyses were conducted using SPSS 21.0, with an α level of 0.05 and effect coefficient of 0.90. All values were expressed as mean ± standard deviation (or standard error), in which the Percent–Percent plot was chosen to determine data normality. The differences among all groups were tested and analyzed for the repeated measurement data based on whether the data were normally distributed (normal distribution: *t* test and ANOVA for continuous variables and χ^2 test for categorical variables; non-normal distribution: Kruskal Wallis H test). $P < 0.05$ was considered to be statistically significant.

RESULTS

Abnormal dietary calcium intake during pregnancy and lactation aggravated development of obesity among male offspring

As shown in Supplementary Figure 2, there were no significant differences in body weight, daily dietary intake, related indexes of glucose (OGTT and ITT) and lipid (TC and TG), and bone Ca/P among the maternal DC, LC, NC and HC groups ($P > 0.05$), with lower bone calcium and phosphorus in the DC and LC groups than in the NC group and higher levels in the HC group ($P < 0.05$), which all proved that the animal model was successful.

Table 2 Effects of maternal different calcium intake on cell cycle and purity of bone marrow mesenchymal stem cells among male offspring

Indicators (%)	NC group (*n* = 9)	DC group (*n* = 9)	LC group (*n* = 9)	HC group (*n* = 9)	χ^2	*P* value
Cell cycle						
G0G1 Phase	91.27 ± 3.14	94.26 ± 2.09	95.08 ± 2.54	92.46 ± 5.01	1.774	0.939
G2M Phase	0.85 ± 0.0091	0.94 ± 0.011	1.05 ± 0.013	0.79 ± 0.0082		
S Phase	7.89 ± 0.81	4.81 ± 0.56	3.87 ± 0.75	6.75 ± 0.94		
Flow cytometry						
Sca-1 (+)	95.03 ± 4.85	98.72 ± 6.12	99.31 ± 5.48	95.49 ± 3.84	4.788	0.188
CD90 (+)	98.12 ± 7.15	99.15 ± 2.04	99.07 ± 8.12	99.31 ± 6.74	0.608	0.895
CD29 (+)	99.13 ± 6.48	99.90 ± 3.46	97.02 ± 8.07	99.62 ± 7.01	0.549	0.908
CD34 (-)	99.08 ± 7.42	96.67 ± 2.93	97.94 ± 1.48	97.29 ± 1.92	0.364	0.546
CD31 (-)	99.46 ± 6.82	99.42 ± 4.12	98.94 ± 1.38	99.26 ± 1.57	0.159	0.690
CD45 (-)	99.79 ± 1.08	99.83 ± 2.54	98.48 ± 1.12	98.65 ± 1.75	0.287	0.963
CD49d (-)	99.13 ± 6.21	98.16 ± 2.32	99.06 ± 2.98	98.11 ± 1.09	5.087	0.166

Four-week-old C57BL/6J female mice were respectively fed with deficient (DC, 0.05%), low (LC, 0.25%), normal (NC, 0.70%) and high (HC,1.20%) calcium reproductive diets for 6 wk before mating and continued their diets throughout gestation and lactation. After weaning, male offspring at 7-day-old from DC, LC, NC and HC groups were chosen to determine the cell cycle and purity of bone marrow mesenchymal stem cells (1×10^6) by flow cytometry. All values were shown as mean ± standard deviation (n = 9/group), and comparisons were made by χ^2 test. NC: Normal-calcium reproductive diet; DC: Deficient-calcium reproductive diet; LC: Low-calcium reproductive diet; HC: High-calcium reproductive diet.

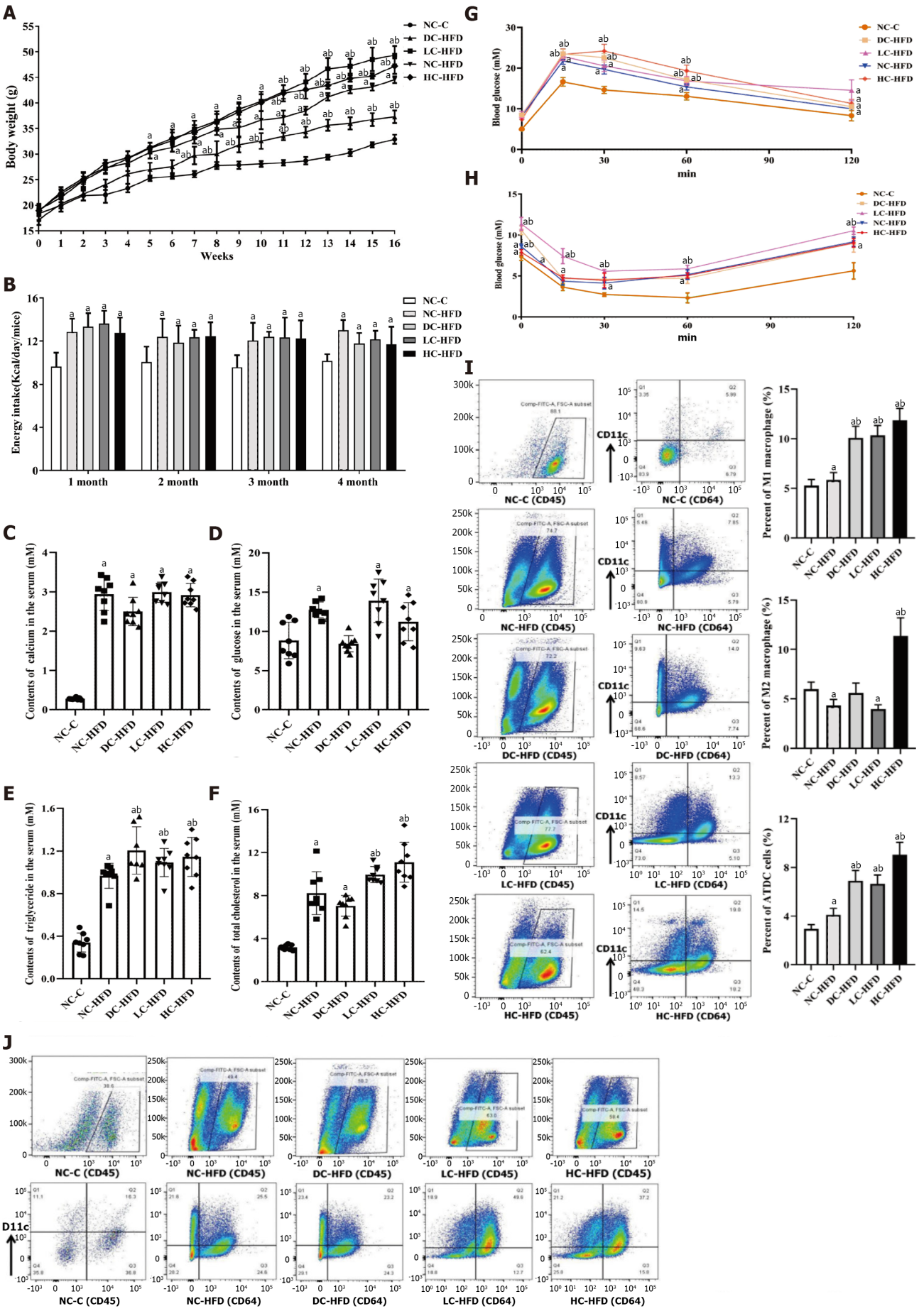
Among the male offspring, the body weight and mental state at weaning were not significantly different among the NC-C, DC-HFD, LC-HFD, NC-HFD and HC-HFD groups ($P > 0.05$, Figure 1A). During the HFD-induced adulthood, body weight (Figure 1A), energy intake (Figure 1B) and concentrations of serum metabolism-related indicators (TG, TC and glucose; Figure 1C-F) were all higher in the HFD groups (DC-HFD, LC-HFD, NC-HFD and HC-HFD) than in the NC-C group ($P < 0.05$). Likewise, the circulating glucose responses to the glucose load, as indicated by OGTT and ITT (Figure 1G and H), showed that there were higher glucose levels in the HFD groups after intraperitoneal glucose administration ($P < 0.05$). Compared with those in the NC-HFD group, maternal low (LC-HFD) and excess (HC-HFD) dietary calcium intake aggravated development of obesity, with significantly higher TC and TG ($P < 0.05$). In contrast, body weight in the DC-HFD group was lower. However, the content of TG was higher than in the NC-HFD group ($P < 0.05$).

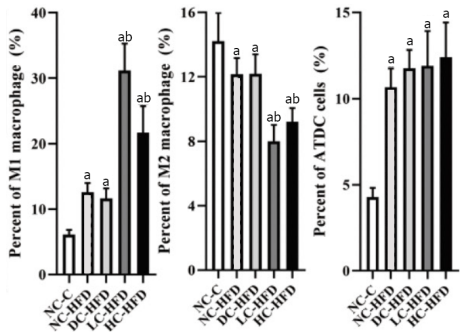
Abnormal dietary calcium intake during pregnancy and lactation caused disorder of infiltration and percentages of immune cells in adipose tissues among male offspring

The percentages of M1 macrophages, M2 macrophages and ATDC cells were demonstrated in eWAT (Figure 1I) and iWAT (Figure 1J). The percentages of M1 macrophages and ATDCs in eWAT were higher, and M2 macrophages were lower in the obese (DC-HFD, LC-HFD, NC-HFD and/or HC-HFD) groups than in the NC-C group ($P < 0.05$). Further comparison among all the HFD groups showed that the percentages of M1 macrophages and ATDCs were increased in the DC-HFD, LC-HFD and HC-HFD groups when compared with the NC-HFD group ($P < 0.05$). In iWAT, the percentages of M1 macrophages and ATDCs were significant higher, and M2 macrophages were lower in the HFD groups than in the NC-C group ($P < 0.05$). Compared with the NC-HFD group, maternal low (LC-HFD) and high (HC-HFD) dietary calcium intake aggravated disorder of M1 and M2 macrophages ($P < 0.05$), which was not found in the DC-HFD group ($P > 0.05$).

Abnormal dietary calcium intake during pregnancy and lactation affected weight and morphology of adipose tissues among male offspring

The weights of eWAT, iWAT, KAT and BAT (Figure 2A) and adipose tissue weight/body weight (Figure 2B) were all higher in the four HFD groups than in the NC-C group ($P < 0.05$). Compared the NC-HFD group, maternal deficient (DC-HFD, eWAT and eWAT/body weight), low (LC-HFD, eWAT, KAT, BAT, eWAT/body weight, KAT/body weight and BAT/body weight) and excess (HC-HFD, BAT and BAT/body weight) dietary calcium intake groups showed increased weights of eWAT, iWAT, KAT and/or BAT, and related adipose tissue weight/body weight ($P < 0.05$). Compared with the NC-C group, the differentiation of adipocytes (number and diameter) in eWAT (Figure 2C and D), iWAT


A
Body weight (g)
Weeks
NC-C
DC-HFD
LC-HFD
NC-HFD
HC-HFD
B
Energy intake(Kcal/day/mice)
1 month
2 month
3 month
4 month
C
Contents of calcium in the serum (mM)
D
Contents of glucose in the serum (mM)
E
Contents of triglyceride in the serum (mM)
F
Contents of total cholesterol in the serum (mM)
G
Blood glucose (mM)
min
H
Blood glucose (mM)
min
I
NC-C (CD45)
NC-C (CD64)
NC-HFD (CD45)
NC-HFD (CD64)
DC-HFD (CD45)
DC-HFD (CD64)
LC-HFD (CD45)
LC-HFD (CD64)
HC-HFD (CD45)
HC-HFD (CD64)
CD11c
Percent of M1 macrophage (%)
Percent of M2 macrophage (%)
Percent of ATDC cells (%)
J
NC-C (CD45)
NC-HFD (CD45)
DC-HFD (CD45)
LC-HFD (CD45)
HC-HFD (CD45)
D11c
NC-C (CD45)
NC-HFD (CD64)
DC-HFD (CD64)
LC-HFD (CD64)
HC-HFD (CD64)



DOI: 10.4252/wjsc.v14.i10.756

Figure 1 Abnormal dietary calcium intake during pregnancy and lactation aggravated development of obesity among male offspring. A: Body weight; B: Energy intake; C: Contents of serum calcium; D: Contents of serum glucose; E: Contents of serum triglyceride; F: Contents of serum total cholesterol; G: Oral glucose tolerance test; H: Insulin glucose tolerance test; I and J: The infiltration and percentages of M1 macrophages, M2 macrophages and ATDC cells in the epididymal white adipose tissue and inguinal white adipose tissue. All pooled data was represented as mean ± standard error (n = 10/group). One-way analysis of variance was performed to compare the differences among the above four groups, and then Student–Newman–Keuls test was used to determine the differences between each two groups. Compared to the NC-C group, $^{a}P < 0.05$. Compared to the NC-HFD group, $^{b}P < 0.05$. NC-C: Normal-calcium reproductive diet and normal-fat diet after weaning; NC-HFD: Normal-calcium reproductive diet and high-fat-diet (HFD) after weaning; DC-HFD: Deficient-calcium reproductive diet and HFD after weaning; LC-HFD: Low-calcium reproductive diet and HFD after weaning; HC-HFD: High-calcium diet and HFD after weaning; eWAT: Epididymal white adipose tissue; iWAT: Inguinal white adipose tissue.

(Figure 2C and E), KAT (Figure 2C and F) and BAT (Figure 2C and G), was more prominent in the DC-HFD, LC-HFD, NC-HFD and HC-HFD groups ($P < 0.05$). Compared with the NC-HFD group, maternal abnormal dietary calcium intake (DC-HFD, LC-HFD and HC-HFD groups) aggravated disorder of proliferation and differentiation of adipocytes in eWAT, iWAT and BAT among male offspring, with larger adipocytes ($P < 0.05$).

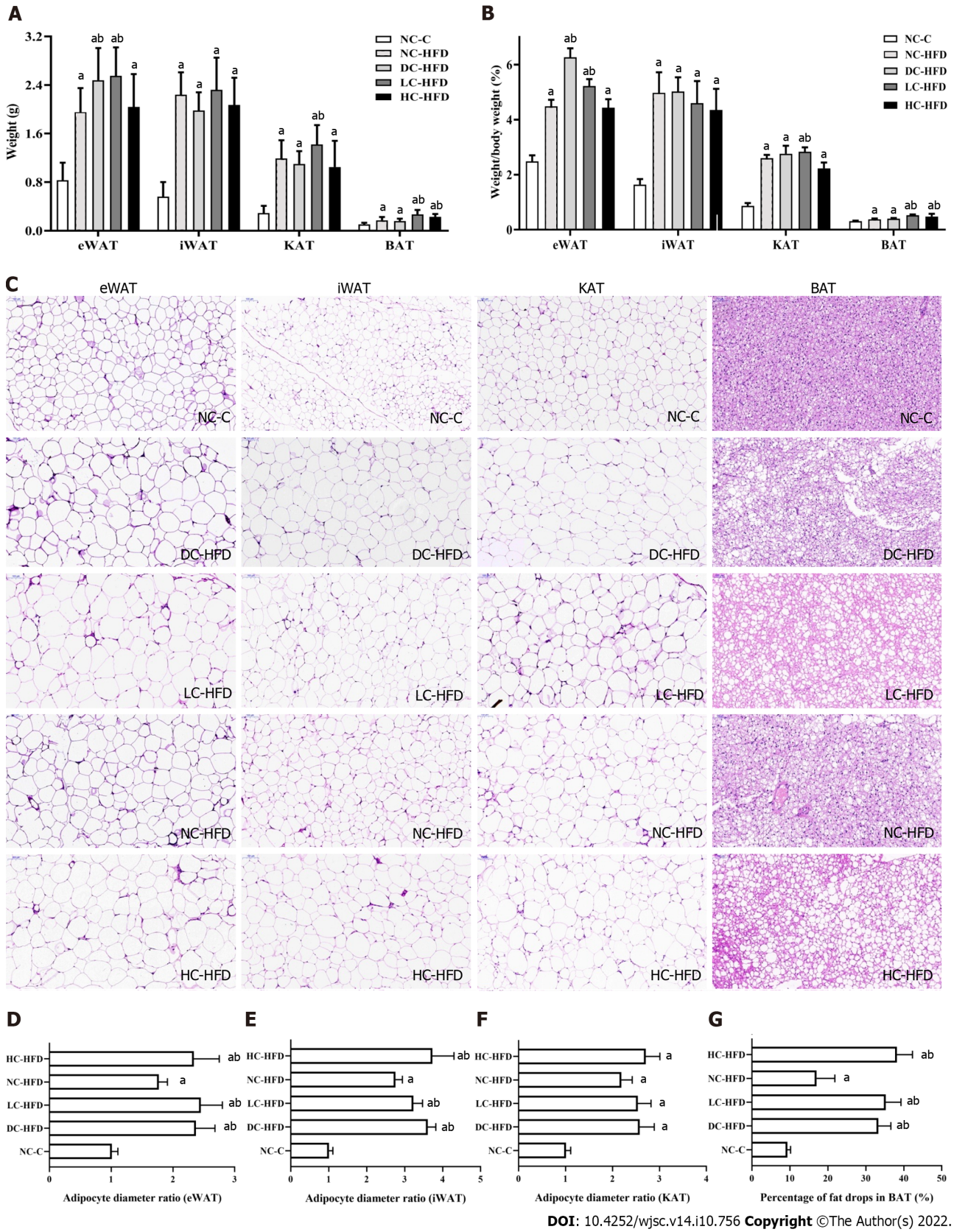
Abnormal dietary calcium intake during pregnancy and lactation regulated target gene expression in adipose tissues among male offspring

As shown in Figure 3, compared with the NC-HFD group, gene expression related to adipogenic differentiation (*PPARγ*, *C/EBPα*, *LPL*, *Fabp4*, *Adiponectin*, *Resistin* and *Leptin*) and Wnt/β-catenin signaling pathway (*Wnt1, Wnt10a, Wnt10b, Wnt5a, CTNNB1, Gsk3β, Axin2* and *TGF7L2*) in eWAT, iWAT, KAT and BAT among the DC-HFD, LC-HFD and HC-HFD groups was more disordered.

Exactly, in eWAT (Figure 3A and B), compared with the NC-HFD group, there were higher expressions of *PPARγ*, *Adiponectin* and *Wnt5a,* and lower expressions of *C/EBPα, CTNNB1* and *TCF7L2* in the DC-HFD group ($P < 0.05$), and higher expressions of *C/EBPα*, *Fabp4*, *LPL*, *Adiponectin* and *Leptin,* and lower expressions of *Resist, CTNNB1* and *TCF7L2* in the LC-HFD group ($P < 0.05$). Higher expressions of *PPARγ*, *C/EBPα*, *LPL*, *Fabp4*, *Adiponectin*, *Leptin* and *Wnt5a,* and lower expressions of *Wnt1, CTNNB1* and *TCF7L2* were demonstrated in the HC-HFD group ($P < 0.05$). In iWAT (Figure 3C and D), expressions of *PPARγ*, *Adiponectin*, *Resistin* and *Leptin* were higher in the DC-HFD, LC-HFD, and HC-HFD groups (with higher *Fabp4* in the HC-HFD) than in the NC-HFD group ($P < 0.05$), with significantly lower expressions of *C/EBPα*, *Wnt1,Wnt10a, Wnt10b, Wnt5a, CTNNB1* and *Gsk3β* in the DC-HFD group ($P < 0.05$), lower expressions of *C/EBPα*, *Wnt1,Wnt10a, Axin2* and *TCF7L2* in the LC-HFD group ($P < 0.05$), and lower expressions of *CTNNB1* and *TCF7L2* in the HC-HFD group ($P < 0.05$). As shown in KAT among the four HFD groups (Figure 3E and F), expressions of *PPARγ*, *LPL*, *Wnt10b* and *Gsk3β* were higher, with the significantly low levels of *Wnt5a, CTNNB1* and *Axin2* in the DC-HFD (accompanied with higher expressions of *Adiponectin* and *Resistin*), LC-HFD (higher expressions of *Adiponectin,* with lower expressions of *C/EBPα* and *Resistin*), and HC-HFD (lower expressions of *Wnt10a* and *Resistin*) groups than in the NC-HFD group ($P < 0.05$). In BAT (Figure 3G and H), expressions of *C/EBPα*, *LPL*, *Adiponectin*, *Resistin, Leptin* and *TGF7L2* were higher, with the significantly lower expressions of *Wnt10a, Wnt5a* and *CTNNB1* in the DC-HFD (lower expressions of *Wnt1*), LC-HFD (higher *Wnt10b* and lower *Gsk3β* expressions), and HC-HFD groups (lower expressions of *Wnt1* and *Gsk3β*) than in the NC-HFD group ($P < 0.05$).

Effects of dietary calcium intake during pregnancy and lactation on adipogenic and osteogenic differentiation potential of BMSCs

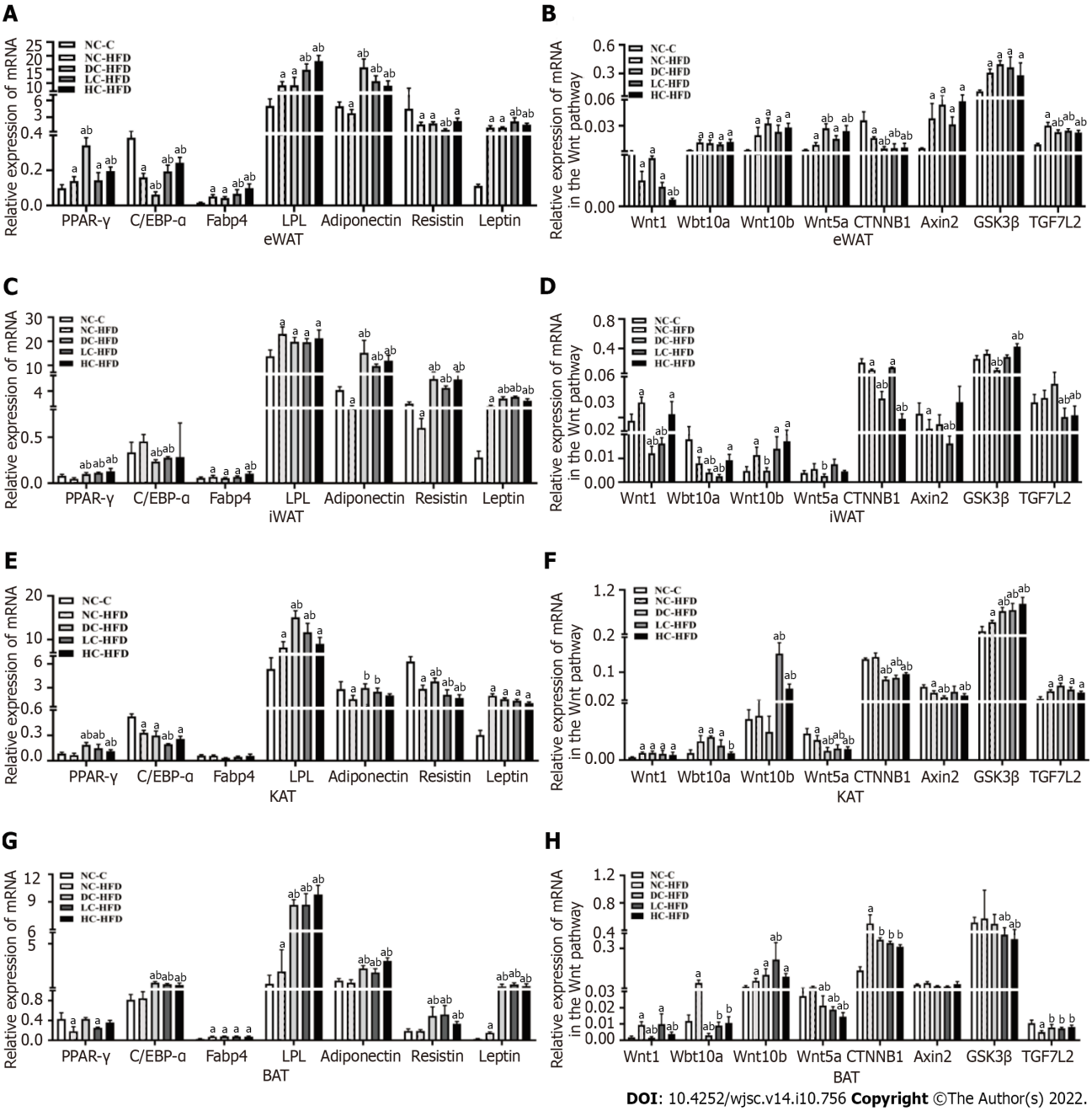
The morphology of BMSCs at P0 (Figure 4A) and P3 generations (Figure 4B) was similar in the DC, LC, NC and HC groups, with no significant differences in the pluripotent stem cells (G0/G1 phase) and purity of BMSCs (Sca-1+, CD90+, CD29+, CD34, CD31, CD45 and CD49d) in the P3 generation ($P > 0.05$, Table 2 and Supplementary Figure 1).



DOI: 10.4252/wjsc.v14.i10.756

Figure 2 Abnormal dietary calcium intake during pregnancy and lactation affected the weight and morphology of adipose tissues among male offspring. A: Weights of the eWAT, iWAT, KAT and BAT; B: Percentage of eWAT, iWAT, KAT and BAT in body weight; C–G: Morphology of adipocytes in eWAT, iWAT, KAT and BAT by hematoxylin and eosin staining. All pooled data was represented as mean ± standard error (n = 10/group). One-way analysis of variance (ANOVA) was performed to compare the differences among the above four groups, and then Student–Newman–Keuls was involved to determine the differences between each two groups. Compared to the NC-C group, $^{a}P < 0.05$. Compared to the NC-HFD group, $^{b}P < 0.05$. NC-C: Normal-calcium reproductive diet and normal-fat diet after weaning; NC-HFD: Normal-calcium reproductive diet and high-fat-diet (HFD) after weaning; DC-HFD: Deficient-calcium reproductive diet and HFD after weaning; LC-HFD: Low-calcium reproductive diet and HFD after weaning; HC-HFD: High-calcium reproductive diet and HFD after weaning; eWAT:

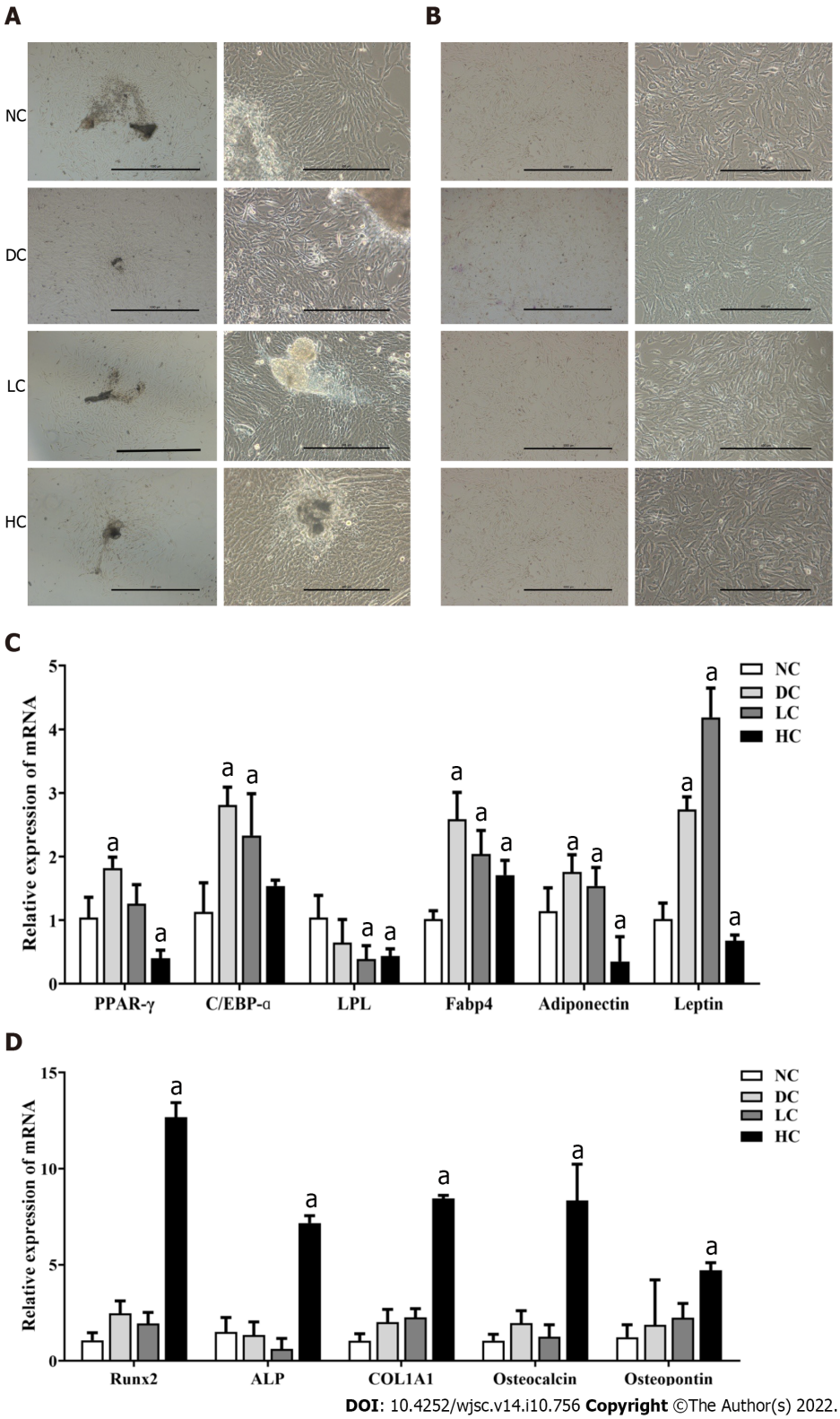
Epididymal white adipose tissue; iWAT: Inguinal white adipose tissue; KAT: Kidney adipose tissue; BAT: Brown adipose tissue.



DOI: 10.4252/wjsc.v14.i10.756

Figure 3 Effects of different dietary calcium intake during pregnancy and lactation on expression of target genes for adipogenic differentiation and Wnt/β-catenin signaling pathway in adipose tissues among male offspring. A, C, E and G: Expression of genes related to adipogenic differentiation in eWAT, iWAT, KAT and BAT; B, D, F and H: Expression of genes related to the Wnt/β-catenin signaling pathway in eWAT, iWAT, KAT and BAT. All data presented as mean ± standard error (n = 10/group). One-way analysis of variance was performed to compare the differences among the above four groups, and then Student–Newman–Keuls test was used to determine the differences between each two groups. Compared to the NC-C group, $^{a}P < 0.05$. Compared to the NC-HFD group, $^{b}P < 0.05$. NC-C: Normal-calcium reproductive diet and normal-fat diet after weaning; NC-HFD: Normal-calcium reproductive diet and high-fat-diet (HFD) after weaning; DC-HFD: Deficient-calcium reproductive diet and HFD after weaning; LC-HFD: Low-calcium reproductive diet and HFD after weaning; HC-HFD: High-calcium reproductive diet and HFD after weaning; eWAT: Epididymal white adipose tissue; iWAT: Inguinal white adipose tissue; KAT: Kidney adipose tissue; BAT: Brown adipose tissue.

In the P3 generation of BMSCs, compared with the NC group, maternal deficient (DC group) and low (LC group) dietary calcium intake promoted adipogenic differentiation potential of BMSCs, with higher levels of *PPARγ*, *C/EBPα*, *Fabp4*, *Adiponectin* and *Leptin* (Figure 4C, $P < 0.05$). Maternal excess dietary calcium intake (HC group) induced osteogenic differentiation and inhibited adipogenic differentiation of BMSCs, with higher levels of *Runx2*, *ALP*, *COL1A1*, *Osteocalcin* and *Osteopontin*, and lower levels of

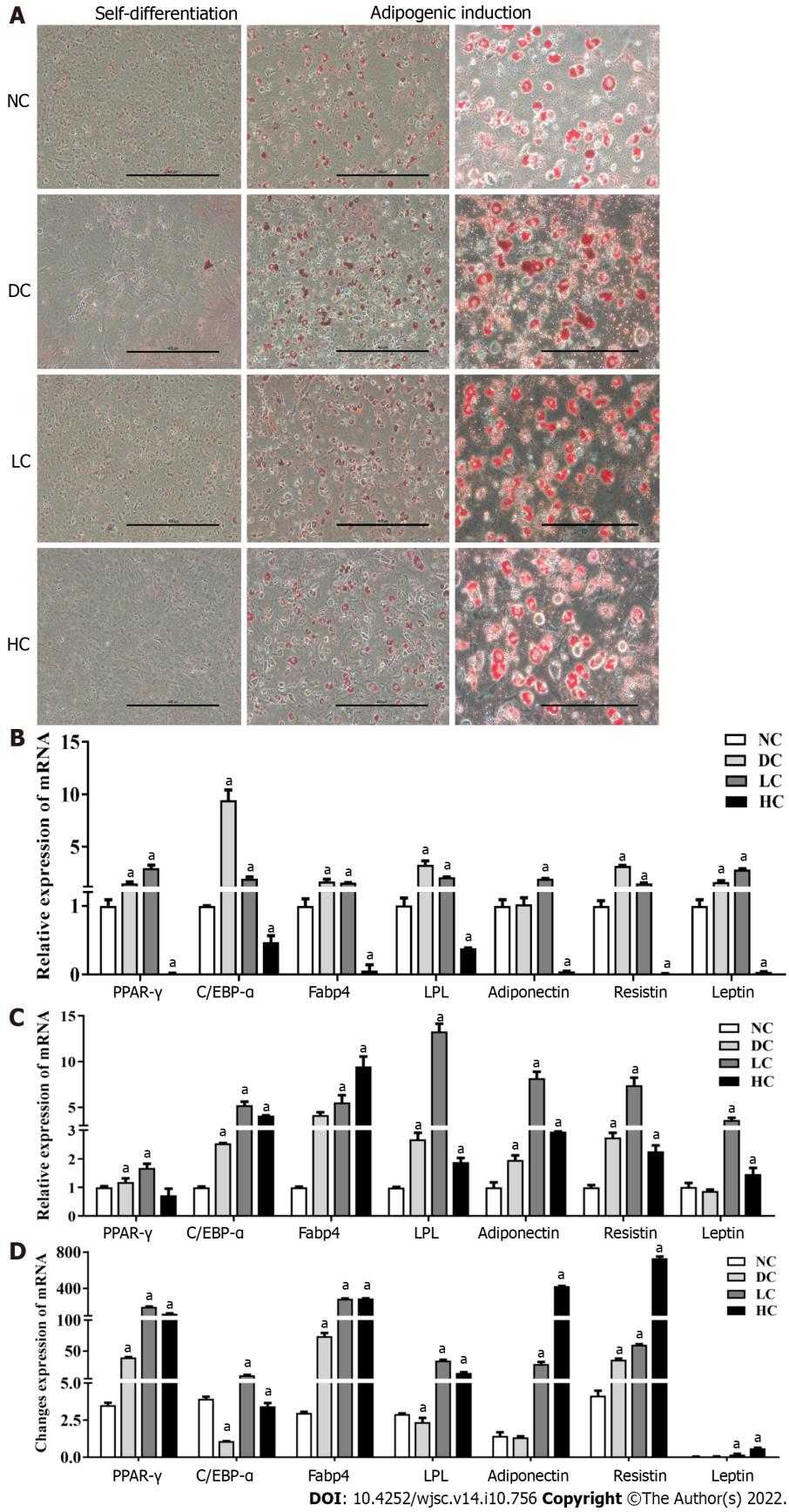


DOI: 10.4252/wjsc.v14.i10.756

Figure 4 Effects of maternal dietary calcium intake during pregnancy and lactation on multi-differentiation potential of bone marrow mesenchymal stem cells among male offspring. A: Morphology of P0 BMSCs; B: Morphology of P3 BMSCs; C: Expression of genes related to adipogenic differentiation; D: Expression of genes related to osteogenic differentiation. All data presented as mean ± standard error (n = 9/ group). One-way analysis of variance was performed to compare the differences among the above four groups, and then Student–Newman–Keuls test was used to determine the differences between each two groups. Compared to the NC group, $^{a}P < 0.05$. NC: Normal-calcium reproductive diet; DC: Deficient-calcium reproductive diet; LC: Low-calcium reproductive diet; HC: High-calcium reproductive diet; BMSCs: Bone marrow mesenchymal stem cells.

PPARγ, *LPL*, *Adiponectin* and *Leptin* (Figure 4C and D, $P < 0.05$).

Under the adipogenic induction (Figure 5), compared with the NC group, maternal abnormal dietary calcium intake (DC, LC and HC groups) promoted adipogenic differentiation of BMSCs, with more lipid



DOI: 10.4252/wjsc.v14.i10.756

Figure 5 Effects of maternal dietary calcium intake during pregnancy and lactation on adipogenic differentiation potential of bone marrow mesenchymal stem cells among male offspring under agent induction. A: Morphology of BMSCs under adipogenic differentiation; B and C: Expression of target genes related to adipogenic differentiation under adipogenic induction and self-differentiation status; D: Ratio of gene expressions between adipogenic differentiation and self-differentiation. All data presented as mean ± standard error (n = 9/group). One-way analysis of variance was performed to compare

the differences among the above four groups, and then Student–Newman–Keuls test was used to determine the differences between each two groups. Compared to the NC group, [a]$P < 0.05$. NC: Normal-calcium reproductive diet; DC: Deficient-calcium reproductive diet; LC: Low-calcium reproductive diet; HC: High-calcium reproductive diet; BMSCs: Bone marrow mesenchymal stem cells.

drops (Figure 5A) and higher expressions of *PPARγ*, *C/EBPα*, *Fabp4*, *LPL*, *Adiponectin*, *Resistin* and *Leptin* (Figure 5C and D). As with expressions of genes related to adipogenic differentiation in the P3 generation (Figure 4C), compared with that in the NC group, expressions of *PPARγ*, *C/EBPα*, *Fabp4*, *LPL*, *Adiponectin*, *Resistin* and *Leptin* in the DC and LC groups were higher under the self-differentiation status (Figure 5B), with lower expressions in the HC group. Under osteogenic induction (Figure 6), compared with the NC group, maternal excess dietary calcium intake (HC group) promoted osteogenic differentiation of BMSCs, with more calcium nodules (Figure 6A) and higher expressions of *Runx2*, *ALP*, *COL1A1* and *Osteocalcin* (Figure 6C and D), which was similar to that under the self-differentiation status (Figure 6B). Furthermore, compared with the NC group, the osteogenic differentiation potential of BMSCs was weaker in the DC and LC groups under osteogenic induction and self-differentiation status (Figure 6A, C and D), with lower expressions of *Runx2*, *ALP*, *COL1A1* and *Osteocalcin*.

Abnormal dietary calcium intake during pregnancy and lactation regulated gene expressions of BMSCs in the Wnt/β-catenin signaling pathway under different interventions

In the P3 generation of BMSCs without induction, compared with the NC group, maternal deficient (DC group) and low (LC group) dietary calcium intake inhibited expressions of *Wnt1, Wnt10a, CTNNB1* and *Axin2* ($P < 0.05$) (Figure 7), while there were significantly higher expressions of *Wnt10b, CTNNB1, Gsk3β* and *TGF7L2,* with lower expressions of *Wnt10a* and *Wnt5a* in the HC group ($P < 0.05$) (Figure 7A).

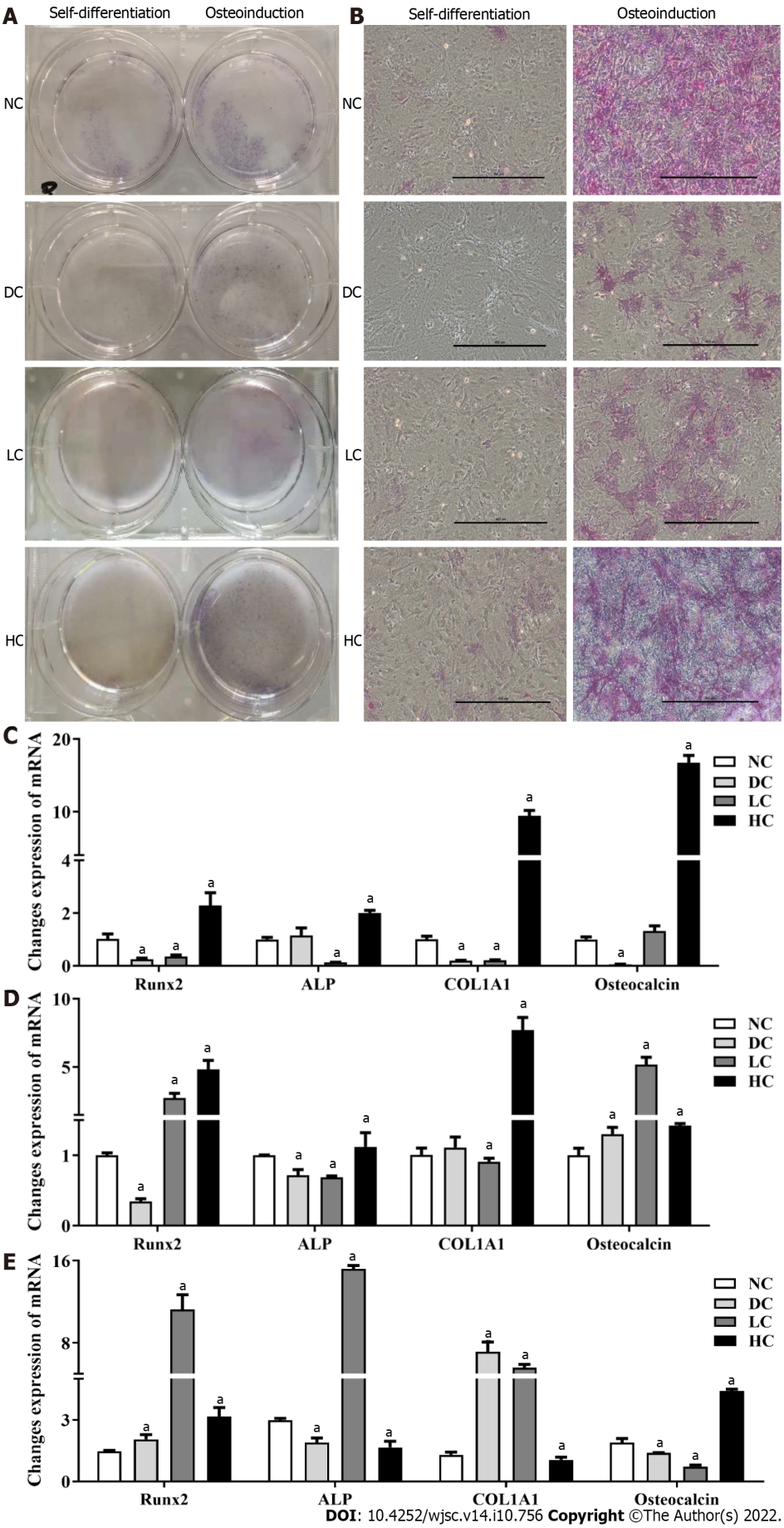
Under adipogenic induction, compared with the NC group, maternal abnormal dietary calcium intake (DC, LC and HC groups) decreased expressions of *Wnt10a, Wnt10b, CTNNB1, Gsk3β* and *TGF7L2* to promote the adipogenic differentiation potential of BMSCs (Figure 7D, $P < 0.05$), while under the self-differentiation status, maternal deficient (DC group) and low (LC group) dietary calcium intake inhibited expressions of *Wnt1, CTNNB1* and *Axin2* ($P < 0.05$), while there were significantly higher levels of *Wnt1, Wnt10a, Wnt10b, Wnt5a, CTNNB1, Axin2, Gsk3β* and *TGF7L2* in the HC group compared with the NC group (Figure 7B, $P < 0.05$), which was consist with the P3 BMSCs.

Under osteogenic induction, compared with the NC group, expressions of *Wnt1, Wnt5a* and *TGF7L2* were lower in the DC group ($P < 0.05$), *Wnt1* expressions was lower in the LC group ($P < 0.05$), and there were significantly higher levels of *Wnt10a, Wnt10b, CTNNB1, Axin2, Gsk3β* and *TGF7L2* in the HC group (Figure 7E, $P < 0.05$). Under the self-differentiation status, maternal DC and LC intake inhibited expressions of *Wnt1, Wnt10a, Wnt5a, CTNNB1* and *TGF7L2* ($P < 0.05$), while there were significantly higher expressions of *Wnt1, Wnt10a, Wnt10b* and *Axin2* in the HC group than in the NC group (Figure 7C, $P < 0.05$).

DISCUSSION

The correlation between the inappropriate consumption of nutrient and occurrence of obesity presents a greatest global public health problem, which needs more novel therapies[30]. A lot of researches have demonstrated that chronically deficient and excessive calcium exposure is as an important contributing factor to the development of obesity by controlling the *de novo* lipogenesis and lipolytic signals through regulating related gene expressions[31-33]. There is also compelling evidence that maternal calcium dysfunction directly affects fat synthesis and metabolism of their offspring[34-37]. In agreement with our findings using a mouse model that maternal inappropriate dietary calcium intake during pregnancy and lactation aggravated development of obesity by elevating cytosolic calcium, with more and larger adipocytes, and disorders of immune cells (M1 macrophages, M2 macrophages and ATDC cells) in the adipose tissues.

It is agreed that the development of obesity is driven by hypertrophy and hyperplasia of the adipocytes in the process of adipogenic differentiation to cause the expansion of fat depots[38]. Lineage-tracing models have shown that the numbers of adipocytes are primarily determined in early life and mostly stable through to adulthood for the remarkable hypertrophic potential of differentiated adipocytes with HFD induction[39-42]. The modulation of cytosolic calcium can regulate the early stage of adipocyte differentiation and thermogenic capacity of BAT in mice[43]. The propensity of adipogenesis to generate new adipocytes in different adipose tissues (eWAT, iWAT, KAT and BAT) highlights the unique characteristics of fat depots. Thus, we should discuss the roles of abnormal dietary calcium intake during pregnancy and lactation on the adipogenic differentiation potential in different adipose tissues among male offspring. This was consistent with our findings that the imbalance of dietary calcium intake in early life could affect the proliferation and differentiation of eWAT, iWAT and BAT, with higher weight of adipose tissue. However, the mechanism remains to be elucidated.



DOI: 10.4252/wjsc.v14.i10.756

Figure 6 Effects of maternal dietary calcium intake during pregnancy and lactation on osteogenic differentiation potential of bone

marrow mesenchymal stem cells among male offspring under agent induction. A and B: Morphology of BMSCs under osteogenic differentiation; C and D: Expression of target genes on osteogenic differentiation under osteogenic induction and self-differentiation status; E: Ratio of gene expressions between osteogenic differentiation and self-differentiation. All data presented as mean ± standard error (n = 9/group). One-way analysis of variance was performed to compare the differences among the above four groups, and then Student–Newman–Keuls test was used to determine the differences between each two groups. Compared to the NC group, $^{a}P < 0.05$. NC: Normal-calcium reproductive diet; DC: Deficient-calcium reproductive diet; LC: Low-calcium reproductive diet; HC: High-calcium reproductive diet; BMSCs: Bone marrow mesenchymal stem cells.

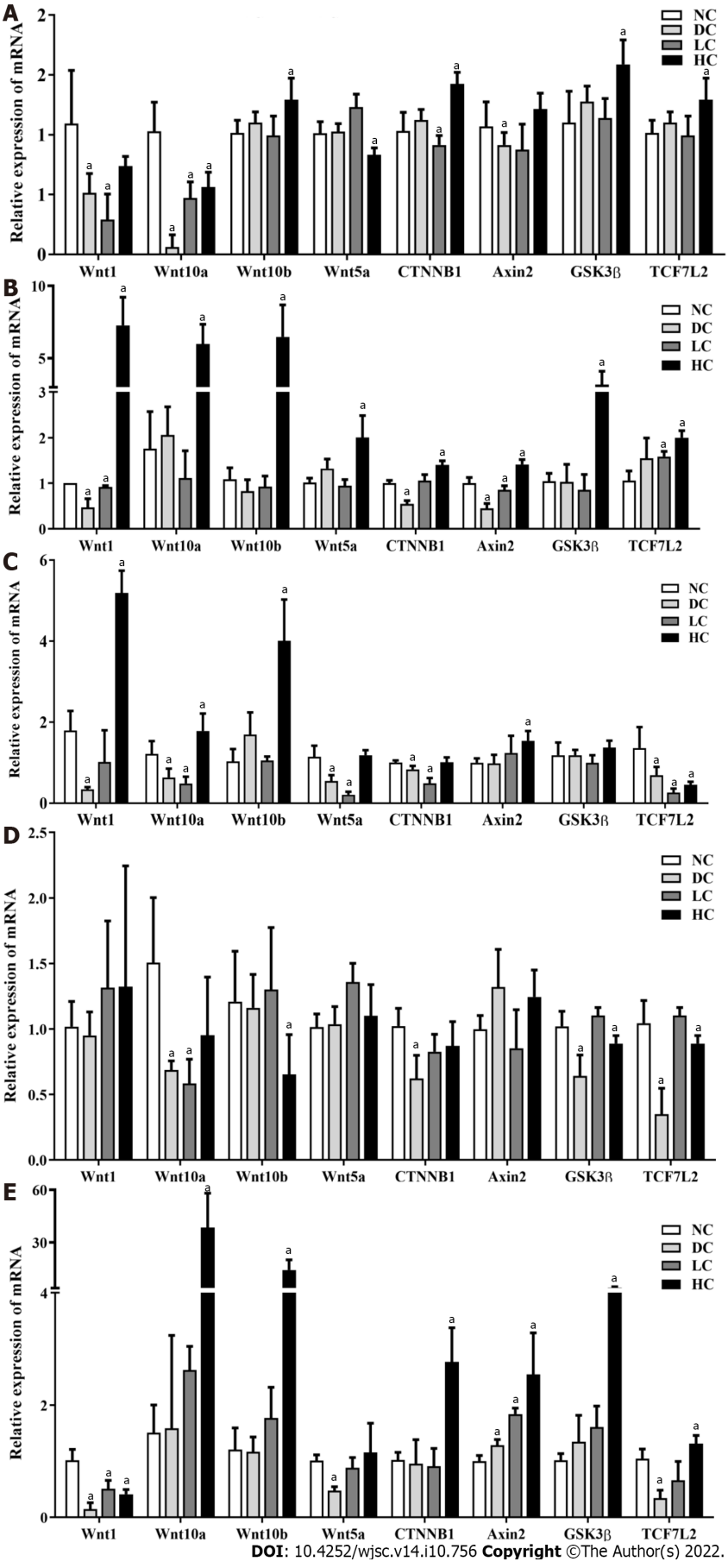
Lineage-tracing studies in animal models have suggested that there are two-step phases in the adipogenic differentiation, including specific preadipocyte formation (MSCs to preadipocytes) and terminal adipocyte maturation (preadipocytes to mature adipocytes), in which the committed preadipocytes from the pluripotent MSCs are activated by a number of critical transcription factors (*PPARγ*, *C/EBPα*, *C/EBPβ* and *FABP4*) and related extracellular signals. At the differentiation of mature adipocytes (second stage), they express all the biomarkers of early adipocyte differentiation as well as the peptide hormones, such as *Adiponectin*, *Resistin*, *Leptin*, *ATGL*, *LPL* and *Perilipin 1*[44,45]. All the above transcription factors are involved in the specific Wnt signaling pathway to affect adipogenic differentiation[46-48]. In our study, abnormal dietary calcium intake during pregnancy and lactation (DC-HFD, LC-HFD and HC-HFD) aggravated expressions of genes related to proliferation and differentiation of adipocytes and Wnt/β-catenin signaling pathway in eWAT, iWAT, KAT or BAT in the adulthood of their male offspring, which could more clearly explain the possible causes for the development of obesity. In the early stage, MSCs, as multipotential progenitor cells, are delicately balanced for their terminal adipo-osteogenic differentiation commitment[49-51]. It has also been reported that this decision process of MSCs is competing and reciprocal, and is precisely achieved by a variety of critical and external cues, including phytocannabinoids, conjugated linoleic acid, calcium, and chemical, physical and biological factors[52-58]. Many investigations *in vitro* had demonstrated that deficient calcium exposure inhibited osteogenesis[59-61]. Conversely, little was known about the effects of inappropriate dietary calcium intake during pregnancy and lactation on adipogenic differentiation, to aggravate the development of obesity in adulthood under HFD induction[55,62]. The major novel finding of our study was that maternal deficient and low dietary calcium intake aggravated the potential adipogenic differentiation and suppressed osteogenic differentiation of BMSCs. Maternal excess dietary calcium intake could play an opposing differentiation role without the exogenous stimuli. In response to reagent induction, both maternal deficient, low and excessive dietary calcium intake could polarize adipogenic differentiation and suppress osteogenic differentiation. All the above results were consistent with the results in the adult offspring with HFD induction.

Terminal differentiation of BMSCs is achieved through a coordinated and highly orchestrated program of triggering different signaling pathways, and activated by various transcription factors that guide the programming alterations of BMSCs to commit the lineage to cause the pathophysiological processes of obesity[63-66]. Thus, it is necessary that our research for screening out the roles of different calcium exposure in early life on the expressions of related transcription factors and signaling pathways could regulate both osteogenic and adipogenic differentiation of BMSCs. This proves that the imbalance of terminal adipo-osteogenic differentiation by abnormal calcium exposure in early life results from the above disorders of gene expressions and Wnt/β-catenin signaling pathway on the differentiation of BMSCs among male offspring.

There were some limitations to this study. Firstly, we required more complex and explicit procedures, including western blotting, to explore the related mechanisms more clearly. Secondly, our conclusions need to be verified in other MSCs and animal models to ensure their feasibility and effectiveness. Finally, the inconsistent results of maternal deficient calcium intake still need to be further discussed.

CONCLUSION

Our results suggest that abnormal dietary calcium intake during gestation and lactation aggravates the development of obesity by programming the adipogenic differentiation potential of BMSCs among male offspring, which is related to the significantly different expressions of target genes for adipogenic differentiation on the Wnt/β-catenin signaling pathway to aggravate dietary-induced obesity in the adulthood. Maternal deficient calcium exposure can inhibit the osteogenic differentiation to cause low body weight. So the importance of this study is that the prevention of adulthood obesity could be moved forward to the appropriate calcium intake in the neonatal period, even the formation of maternal germ cells and fertilized egg.



DOI: 10.4252/wjsc.v14.i10.756

Figure 7 Effects of maternal different dietary calcium intake on gene expressions of bone marrow mesenchymal stem cells at the Wnt/β-catenin signaling pathway under different interventions among male offspring. A: P3 BMSCs; B and D: Adipogenic induction and self-differentiation

status of BMSCs; C and E: Osteogenic induction and self-differentiation status of BMSCs. All data presented as mean ± standard error (n = 9/group). One-way analysis of variance was performed to compare the differences among the above four groups, and then Student–Newman–Keuls test was used to determine the differences between each two groups. Compared to the NC group, $^{a}P < 0.05$. NC: Normal-calcium reproductive diet; DC: Deficient-calcium reproductive diet; LC: Low-calcium reproductive diet; HC: High-calcium reproductive diet; BMSCs: Bone marrow mesenchymal stem cells.

ARTICLE HIGHLIGHTS

Research background

Obesity is characterized by the hypertrophy and hyperplasia of adipocytes, in which the commitment from bone mesenchymal stem cells (BMSCs) to preadipocytes is the important process for their hyperplasia. Our previous study showed that dietary insufficient and excessive calcium intake during pregnancy and lactation increased the body weight of offspring, using a high-fat-diet-induced obese mouse model and epidemiological cohorts. However, whether maternal inappropriate dietary calcium intake could affect the adipogenic differentiation potential of MSCs is still unclear.

Research motivation

This study was designed to investigate the effects of abnormal dietary calcium intake during gestation and lactation on the muti-differentiation potential of BMSCs among male offspring, and explore the possible role of the Wnt/β-catenin signaling pathway, which might aggravate the development of obesity, with more excessive lipid accumulation in adulthood.

Research objectives

We presented the possibility that abnormal dietary calcium intake during pregnancy and lactation could derive hyperplasic adipogenesis from BMSCs by regulating target gene expressions profiles through the fetus to adulthood among their male offspring.

Research methods

Four-week-old female C57BL/6N mice were fed by deficient, low, normal and excessive calcium reproductive diets throughout pregnancy and lactation. The BMSCs were obtained from 7-day-old male offspring to measure their adipogenic differentiation potential through the Wnt/β-catenin signaling pathway. The other weaning male pups were fed a high-fat diet for 16 wk along with a normal-fat diet as the control. Serum was collected for biochemical analysis. Adipose tissues were excised for histological examination, immunohistochemistry, determining the proportions of immune cells by flow cytometry, and gene expressions related to adipogenic differentiation and Wnt/β-catenin signaling pathway by real-time reverse transcription polymerase chain reaction.

Research results

Maternal deficient, low and excess dietary calcium intake aggravated dietary-induced obesity with more/larger adipocytes and higher serum metabolism indicators, along with disordered expressions of genes related to adipogenic differentiation (*PPARγ, C/EBPα, Fabp4, LPL, Adiponectin, Resistin* and *Leptin*) in the adipose tissues among the male offspring. We also showed significantly different expressions of similarly specific genes in BMSCs to successfully polarize adipogenic differentiation and suppress osteogenic differentiation *in vivo* and *in vitro*, respectively. The related mechanistic insights were gained to worsen this adipogenic differentiation through the Wnt/β-catenin signaling pathway in the BMSCs and adult adipose tissues.

Research conclusions

Abnormal dietary calcium intake during pregnancy and lactation might program the adipogenic differentiation potential of BMSCs among male offspring, which was related to the significantly different expressions of target genes in the Wnt/β-catenin signaling pathway to preserve more adipocytes to aggravate dietary-induced obesity in adulthood.

Research perspectives

The importance of this study is that the prevention of adulthood obesity could be moved forward to the appropriate calcium intake in the neonatal period, even the formation of maternal germ cells and fertilized egg.

ACKNOWLEDGEMENTS

We would like to thank Yuan-Lin Liu and Yi Zhang for their helps on the experiments of BMSCs and

Servicebio Co. Ltd. for their morphological analysis service.

FOOTNOTES

Author contributions: Li P and Wang Y designed the study, performed the data analysis and wrote the manuscript; Li P, Chen XY and Tang TT were responsible for all the animal procedures and experiments; Liu YL and Liu WJ collected the bone mesenchymal stem cells; Qi KM and Zhang Y supervised the final manuscript.

Supported by National Natural Science Foundation of China (to P.L.), No. 81602859 and No. 82173524; and National Key Research and Development Program of China (to Y.Z.), No. 2016YFC1000305.

Institutional review board statement: This study was reviewed and approved by the institutional review board statement at Beijing Children's Hospital, Capital Medical University, National Center for Children's Health.

Institutional animal care and use committee statement: All animal studies were approved and conducted in accordance with the Beijing Academy of Military Medical Sciences Guide for the Care and Usage Committee of Laboratory Animals. Meanwhile, the animal care and use committee statement used in this study was approved on the Ethics of Animal Experiments of Academy of Military Medical Sciences in China, No. IACUC-DWZX-2019-704.

Conflict-of-interest statement: All the authors report no relevant conflicts of interest for this article.

Data sharing statement: The data and materials that support the findings of this study are available from the corresponding author upon the reasonable requests.

ARRIVE guidelines statement: The authors have read the ARRIVE guidelines, and the manuscript was prepared and revised according to the ARRIVE guidelines.

Country/Territory of origin: China

ORCID number: Yi Zhang 0000-0003-2498-3948.

S-Editor: Fan JR
L-Editor: Kerr C
P-Editor: Zhang XD

REFERENCES

1 **Chooi YC**, Ding C, Magkos F. The epidemiology of obesity. *Metabolism* 2019; **92**: 6-10 [PMID: 30253139 DOI: 10.1016/j.metabol.2018.09.005]
2 **Lavie CJ**, Pandey A, Lau DH, Alpert MA, Sanders P. Obesity and Atrial Fibrillation Prevalence, Pathogenesis, and Prognosis: Effects of Weight Loss and Exercise. *J Am Coll Cardiol* 2017; **70**: 2022-2035 [PMID: 29025560 DOI: 10.1016/j.jacc.2017.09.002]
3 **Rivera JÁ**, de Cossío TG, Pedraza LS, Aburto TC, Sánchez TG, Martorell R. Childhood and adolescent overweight and obesity in Latin America: a systematic review. *Lancet Diabetes Endocrinol* 2014; **2**: 321-332 [PMID: 24703050 DOI: 10.1016/S2213-8587(13)70173-6]
4 **Tencerova M**, Figeac F, Ditzel N, Taipaleenmäki H, Nielsen TK, Kassem M. High-Fat Diet-Induced Obesity Promotes Expansion of Bone Marrow Adipose Tissue and Impairs Skeletal Stem Cell Functions in Mice. *J Bone Miner Res* 2018; **33**: 1154-1165 [PMID: 29444341 DOI: 10.1002/jbmr.3408]
5 **Li SN**, Wu JF. TGF-β/SMAD signaling regulation of mesenchymal stem cells in adipocyte commitment. *Stem Cell Res Ther* 2020; **11**: 41 [PMID: 31996252 DOI: 10.1186/s13287-020-1552-y]
6 **Ambrosi TH**, Scialdone A, Graja A, Gohlke S, Jank AM, Bocian C, Woelk L, Fan H, Logan DW, Schürmann A, Saraiva LR, Schulz TJ. Adipocyte Accumulation in the Bone Marrow during Obesity and Aging Impairs Stem Cell-Based Hematopoietic and Bone Regeneration. *Cell Stem Cell* 2017; **20**: 771-784.e6 [PMID: 28330582 DOI: 10.1016/j.stem.2017.02.009]
7 **Lin YH**, Kang L, Feng WH, Cheng TL, Tsai WC, Huang HT, Lee HC, Chen CH. Effects of Lipids and Lipoproteins on Mesenchymal Stem Cells Used in Cardiac Tissue Regeneration. *Int J Mol Sci* 2020; **21** [PMID: 32635662 DOI: 10.3390/ijms21134770]
8 **Louwen F**, Ritter A, Kreis NN, Yuan J. Insight into the development of obesity: functional alterations of adipose-derived mesenchymal stem cells. *Obes Rev* 2018; **19**: 888-904 [PMID: 29521029 DOI: 10.1111/obr.12679]

9 **Cristancho AG**, Lazar MA. Forming functional fat: a growing understanding of adipocyte differentiation. *Nat Rev Mol Cell Biol* 2011; **12**: 722-734 [PMID: 21952300 DOI: 10.1038/nrm3198]

10 **Porro S**, Genchi VA, Cignarelli A, Natalicchio A, Laviola L, Giorgino F, Perrini S. Dysmetabolic adipose tissue in obesity: morphological and functional characteristics of adipose stem cells and mature adipocytes in healthy and unhealthy obese subjects. *J Endocrinol Invest* 2021; **44**: 921-941 [PMID: 33145726 DOI: 10.1007/s40618-020-01446-8]

11 **Rosen ED**, MacDougald OA. Adipocyte differentiation from the inside out. *Nat Rev Mol Cell Biol* 2006; **7**: 885-896 [PMID: 17139329 DOI: 10.1038/nrm2066]

12 **Matsushita K**, Dzau VJ. Mesenchymal stem cells in obesity: insights for translational applications. *Lab Invest* 2017; **97**: 1158-1166 [PMID: 28414326 DOI: 10.1038/labinvest.2017.42]

13 **Zhang P**, Zhang H, Lin J, Xiao T, Xu R, Fu Y, Zhang Y, Du Y, Cheng J, Jiang H. Insulin impedes osteogenesis of BMSCs by inhibiting autophagy and promoting premature senescence *via* the TGF-β1 pathway. *Aging (Albany NY)* 2020; **12**: 2084-2100 [PMID: 32017705 DOI: 10.18632/aging.102723]

14 **Goldstein RF**, Abell SK, Ranasinha S, Misso M, Boyle JA, Black MH, Li N, Hu G, Corrado F, Rode L, Kim YJ, Haugen M, Song WO, Kim MH, Bogaerts A, Devlieger R, Chung JH, Teede HJ. Association of Gestational Weight Gain With Maternal and Infant Outcomes: A Systematic Review and Meta-analysis. *JAMA* 2017; **317**: 2207-2225 [PMID: 28586887 DOI: 10.1001/jama.2017.3635]

15 **Gingras V**, Hivert MF, Oken E. Early-Life Exposures and Risk of Diabetes Mellitus and Obesity. *Curr Diab Rep* 2018; **18**: 89 [PMID: 30159823 DOI: 10.1007/s11892-018-1050-0]

16 **Hoffman DJ**, Reynolds RM, Hardy DB. Developmental origins of health and disease: current knowledge and potential mechanisms. *Nutr Rev* 2017; **75**: 951-970 [PMID: 29186623 DOI: 10.1093/nutrit/nux053]

17 **Soares MJ**, Pathak K, Calton EK. Calcium and vitamin D in the regulation of energy balance: where do we stand? *Int J Mol Sci* 2014; **15**: 4938-4945 [PMID: 24658438 DOI: 10.3390/ijms15034938]

18 **Li P**, Fan C, Lu Y, Qi K. Effects of calcium supplementation on body weight: a meta-analysis. *Am J Clin Nutr* 2016; **104**: 1263-1273 [PMID: 27733391 DOI: 10.3945/ajcn.116.136242]

19 **Zemel MB**, Miller SL. Dietary calcium and dairy modulation of adiposity and obesity risk. *Nutr Rev* 2004; **62**: 125-131 [PMID: 15141427 DOI: 10.1111/j.1753-4887.2004.tb00034.x]

20 **Gernand AD**, Schulze KJ, Stewart CP, West KP Jr, Christian P. Micronutrient deficiencies in pregnancy worldwide: health effects and prevention. *Nat Rev Endocrinol* 2016; **12**: 274-289 [PMID: 27032981 DOI: 10.1038/nrendo.2016.37]

21 **Bolland MJ**, Leung W, Tai V, Bastin S, Gamble GD, Grey A, Reid IR. Calcium intake and risk of fracture: systematic review. *BMJ* 2015; **351**: h4580 [PMID: 26420387 DOI: 10.1136/bmj.h4580]

22 **Li P**, Tang T, Chang X, Fan X, Chen X, Wang R, Fan C, Qi K. Abnormality in Maternal Dietary Calcium Intake During Pregnancy and Lactation Promotes Body Weight Gain by Affecting the Gut Microbiota in Mouse Offspring. *Mol Nutr Food Res* 2019; **63**: e1800399 [PMID: 30576063 DOI: 10.1002/mnfr.201800399]

23 **Li P**, Yan K, Chang X, Chen X, Wang R, Fan X, Tang T, Zhan D, Qi K. Sex-specific maternal calcium requirements for the prevention of nonalcoholic fatty liver disease by altering the intestinal microbiota and lipid metabolism in the high-fat-diet-fed offspring mice. *Gut Microbes* 2020; **11**: 1590-1607 [PMID: 32576050 DOI: 10.1080/19490976.2020.1768645]

24 **Chang XL**, Shang Y, Liu YJ, Li P, Wang YY, Liang AM, Qi KM. Effects of calcium supplementation during the pregnancy and early infancy stage on the body mass index and gut microbiota in the infants. *Chinese journal of preventive medicine* 2018; **52**: 642-646 [PMID: 29886687 DOI: 10.3760/cma.j.issn.0253-9624.2018.06.014]

25 **Chen XR**, Bai J, Yuan SJ, Yu CX, Huang J, Zhang TL, Wang K. Calcium phosphate nanoparticles are associated with inorganic phosphate-induced osteogenic differentiation of rat bone marrow stromal cells. *Chem Biol Interact* 2015; **238**: 111-117 [PMID: 26111760 DOI: 10.1016/j.cbi.2015.06.027]

26 **Lei Q**, Chen J, Huang W, Wu D, Lin H, Lai Y. Proteomic analysis of the effect of extracellular calcium ions on human mesenchymal stem cells: Implications for bone tissue engineering. *Chem Biol Interact* 2015; **233**: 139-146 [PMID: 25824407 DOI: 10.1016/j.cbi.2015.03.021]

27 **Jothimani G**, Di Liddo R, Pathak S, Piccione M, Sriramulu S, Banerjee A. Wnt signaling regulates the proliferation potential and lineage commitment of human umbilical cord derived mesenchymal stem cells. *Mol Biol Rep* 2020; **47**: 1293-1308 [PMID: 31853765 DOI: 10.1007/s11033-019-05232-5]

28 **Park JS**, Kim M, Song NJ, Kim JH, Seo D, Lee JH, Jung SM, Lee JY, Lee J, Lee YS, Park KW, Park SH. A Reciprocal Role of the Smad4-Taz Axis in Osteogenesis and Adipogenesis of Mesenchymal Stem Cells. *Stem Cells* 2019; **37**: 368-381 [PMID: 30444564 DOI: 10.1002/stem.2949]

29 **Liu W**, Zhou N, Liu Y, Zhang W, Li X, Wang Y, Zheng R, Zhang Y. Mesenchymal stem cell exosome-derived miR-223 alleviates acute graft-versus-host disease *via* reducing the migration of donor T cells. *Stem Cell Res Ther* 2021; **12**: 153 [PMID: 33637123 DOI: 10.1186/s13287-021-02159-2]

30 **Gao P**, Jiang Y, Wu H, Sun F, Li Y, He H, Wang B, Lu Z, Hu Y, Wei X, Cui Y, He C, Wang L, Zheng H, Yang G, Liu D, Yan Z, Zhu Z. Inhibition of Mitochondrial Calcium Overload by SIRT3 Prevents Obesity- or Age-Related Whitening of Brown Adipose Tissue. *Diabetes* 2020; **69**: 165-180 [PMID: 31712319 DOI: 10.2337/db19-0526]

31 **Fu S**, Yang L, Li P, Hofmann O, Dicker L, Hide W, Lin X, Watkins SM, Ivanov AR, Hotamisligil GS. Aberrant lipid metabolism disrupts calcium homeostasis causing liver endoplasmic reticulum stress in obesity. *Nature* 2011; **473**: 528-531 [PMID: 21532591 DOI: 10.1038/nature09968]

32 **Yang TT**, Suk HY, Yang X, Olabisi O, Yu RY, Durand J, Jelicks LA, Kim JY, Scherer PE, Wang Y, Feng Y, Rossetti L, Graef IA, Crabtree GR, Chow CW. Role of transcription factor NFAT in glucose and insulin homeostasis. *Mol Cell Biol* 2006; **26**: 7372-7387 [PMID: 16908540 DOI: 10.1128/MCB.00580-06]

33 **Hotamisligil GS**. Inflammation and metabolic disorders. *Nature* 2006; **444**: 860-867 [PMID: 17167474 DOI: 10.1038/nature05485]

34 **Marangoni F**, Cetin I, Verduci E, Canzone G, Giovannini M, Scollo P, Corsello G, Poli A. Maternal Diet and Nutrient Requirements in Pregnancy and Breastfeeding. An Italian Consensus Document. *Nutrients* 2016; **8** [PMID: 27754423 DOI: 10.3390/nu8100629]

35 **Wang Q**, Zhu C, Sun M, Maimaiti R, Ford SP, Nathanielsz PW, Ren J, Guo W. Maternal obesity impairs fetal cardiomyocyte contractile function in sheep. *FASEB J* 2019; **33**: 2587-2598 [PMID: 30289749 DOI: 10.1096/fj.201800988R]

36 **Ng SF**, Lin RC, Laybutt DR, Barres R, Owens JA, Morris MJ. Chronic high-fat diet in fathers programs β-cell dysfunction in female rat offspring. *Nature* 2010; **467**: 963-966 [PMID: 20962845 DOI: 10.1038/nature09491]

37 **Marotte C**, Bryk G, Gonzales Chaves MM, Lifshitz F, de Portela ML, Zeni SN. Low dietary calcium and obesity: a comparative study in genetically obese and normal rats during early growth. *Eur J Nutr* 2014; **53**: 769-778 [PMID: 24061348 DOI: 10.1007/s00394-013-0581-z]

38 **Ghaben AL**, Scherer PE. Adipogenesis and metabolic health. *Nat Rev Mol Cell Biol* 2019; **20**: 242-258 [PMID: 30610207 DOI: 10.1038/s41580-018-0093-z]

39 **Wang QA**, Tao C, Gupta RK, Scherer PE. Tracking adipogenesis during white adipose tissue development, expansion and regeneration. *Nat Med* 2013; **19**: 1338-1344 [PMID: 23995282 DOI: 10.1038/nm.3324]

40 **Spalding KL**, Arner E, Westermark PO, Bernard S, Buchholz BA, Bergmann O, Blomqvist L, Hoffstedt J, Näslund E, Britton T, Concha H, Hassan M, Rydén M, Frisén J, Arner P. Dynamics of fat cell turnover in humans. *Nature* 2008; **453**: 783-787 [PMID: 18454136 DOI: 10.1038/nature06902]

41 **Lin H**, An Y, Tang H, Wang Y. Alterations of Bile Acids and Gut Microbiota in Obesity Induced by High Fat Diet in Rat Model. *J Agric Food Chem* 2019; **67**: 3624-3632 [PMID: 30832480 DOI: 10.1021/acs.jafc.9b00249]

42 **Vishvanath L**, MacPherson KA, Hepler C, Wang QA, Shao M, Spurgin SB, Wang MY, Kusminski CM, Morley TS, Gupta RK. Pdgfrβ+ Mural Preadipocytes Contribute to Adipocyte Hyperplasia Induced by High-Fat-Diet Feeding and Prolonged Cold Exposure in Adult Mice. *Cell Metab* 2016; **23**: 350-359 [PMID: 26626462 DOI: 10.1016/j.cmet.2015.10.018]

43 **Knittle JL**, Timmers K, Ginsberg-Fellner F, Brown RE, Katz DP. The growth of adipose tissue in children and adolescents. Cross-sectional and longitudinal studies of adipose cell number and size. *J Clin Invest* 1979; **63**: 239-246 [PMID: 429551 DOI: 10.1172/JCI109295]

44 **Merrick D**, Sakers A, Irgebay Z, Okada C, Calvert C, Morley MP, Percec I, Seale P. Identification of a mesenchymal progenitor cell hierarchy in adipose tissue. *Science* 2019; **364** [PMID: 31023895 DOI: 10.1126/science.aav2501]

45 **Rosen ED**, Sarraf P, Troy AE, Bradwin G, Moore K, Milstone DS, Spiegelman BM, Mortensen RM. PPAR gamma is required for the differentiation of adipose tissue *in vivo* and in vitro. *Mol Cell* 1999; **4**: 611-617 [PMID: 10549292 DOI: 10.1016/s1097-2765(00)80211-7]

46 **Farmer SR**. Transcriptional control of adipocyte formation. *Cell Metab* 2006; **4**: 263-273 [PMID: 17011499 DOI: 10.1016/j.cmet.2006.07.001]

47 **Aamir K**, Khan HU, Sethi G, Hossain MA, Arya A. Wnt signaling mediates TLR pathway and promote unrestrained adipogenesis and metaflammation: Therapeutic targets for obesity and type 2 diabetes. *Pharmacol Res* 2020; **152**: 104602 [PMID: 31846761 DOI: 10.1016/j.phrs.2019.104602]

48 **Chen X**, Ayala I, Shannon C, Fourcaudot M, Acharya NK, Jenkinson CP, Heikkinen S, Norton L. The Diabetes Gene and Wnt Pathway Effector TCF7L2 Regulates Adipocyte Development and Function. *Diabetes* 2018; **67**: 554-568 [PMID: 29317436 DOI: 10.2337/db17-0318]

49 **Chen Q**, Shou P, Zheng C, Jiang M, Cao G, Yang Q, Cao J, Xie N, Velletri T, Zhang X, Xu C, Zhang L, Yang H, Hou J, Wang Y, Shi Y. Fate decision of mesenchymal stem cells: adipocytes or osteoblasts? *Cell Death Differ* 2016; **23**: 1128-1139 [PMID: 26868907 DOI: 10.1038/cdd.2015.168]

50 **Woeller CF**, Lim SA, Roztocil E, Yee M, Beier EE, Puzas JE, O'Reilly MA. Neonatal hyperoxia impairs adipogenesis of bone marrow-derived mesenchymal stem cells and fat accumulation in adult mice. *Free Radic Biol Med* 2021; **167**: 287-298 [PMID: 33757863 DOI: 10.1016/j.freeradbiomed.2021.03.005]

51 **Ishikane S**, Ikushima E, Igawa K, Tomooka K, Takahashi-Yanaga F. Differentiation-inducing factor-1 potentiates adipogenic differentiation and attenuates the osteogenic differentiation of bone marrow-derived mesenchymal stem cells. *Biochim Biophys Acta Mol Cell Res* 2021; **1868**: 118909 [PMID: 33189784 DOI: 10.1016/j.bbamcr.2020.118909]

52 **Oh JH**, Karadeniz F, Lee JI, Seo Y, Kong CS. *Ligustrum japonicum* Thunb. Fruits Exert Antiosteoporotic Properties in Bone Marrow-Derived Mesenchymal Stromal Cells *via* Regulation of Adipocyte and Osteoblast Differentiation. *Stem Cells Int* 2021; **2021**: 8851884 [PMID: 33628272 DOI: 10.1155/2021/8851884]

53 **Baer PC**, Koch B, Hickmann E, Schubert R, Cinatl J Jr, Hauser IA, Geiger H. Isolation, Characterization, Differentiation and Immunomodulatory Capacity of Mesenchymal Stromal/Stem Cells from Human Perirenal Adipose Tissue. *Cells* 2019; **8** [PMID: 31671899 DOI: 10.3390/cells8111346]

54 **Qu P**, Wang L, Min Y, McKennett L, Keller JR, Lin PC. Vav1 Regulates Mesenchymal Stem Cell Differentiation Decision Between Adipocyte and Chondrocyte *via* Sirt1. *Stem Cells* 2016; **34**: 1934-1946 [PMID: 26990002 DOI: 10.1002/stem.2365]

55 **Fan J**, Lee CS, Kim S, Zhang X, Pi-Anfruns J, Guo M, Chen C, Rahnama M, Li J, Wu BM, Aghaloo TL, Lee M. Trb3 controls mesenchymal stem cell lineage fate and enhances bone regeneration by scaffold-mediated local gene delivery. *Biomaterials* 2021; **264**: 120445 [PMID: 33069136 DOI: 10.1016/j.biomaterials.2020.120445]

56 **Li Y**, Stahl CH. Dietary calcium deficiency and excess both impact bone development and mesenchymal stem cell lineage priming in neonatal piglets. *J Nutr* 2014; **144**: 1935-1942 [PMID: 25320190 DOI: 10.3945/jn.114.194787]

57 **Casado-Díaz A**, Anter J, Müller S, Winter P, Quesada-Gómez JM, Dorado G. Transcriptomic Analyses of Adipocyte Differentiation From Human Mesenchymal Stromal-Cells (MSC). *J Cell Physiol* 2017; **232**: 771-784 [PMID: 27349923 DOI: 10.1002/jcp.25472]

58 **Fellous T**, De Maio F, Kalkan H, Carannante B, Boccella S, Petrosino S, Maione S, Di Marzo V, Iannotti FA. Phytocannabinoids promote viability and functional adipogenesis of bone marrow-derived mesenchymal stem cells through different molecular targets. *Biochem Pharmacol* 2020; **175**: 113859 [PMID: 32061773 DOI: 10.1016/j.bcp.2020.113859]

59 **Platt ID**, El-Sohemy A. Regulation of osteoblast and adipocyte differentiation from human mesenchymal stem cells by conjugated linoleic acid. *J Nutr Biochem* 2009; **20**: 956-964 [PMID: 19019668 DOI: 10.1016/j.jnutbio.2008.08.008]

60 **Ye J**, Gong P. NGF-CS/HA-coating composite titanium facilitates the differentiation of bone marrow mesenchymal stem cells into osteoblast and neural cells. *Biochem Biophys Res Commun* 2020; **531**: 290-296 [PMID: 32800542 DOI:

10.1016/j.bbrc.2020.06.158]

61 **Salehi A**, Mobarhan MA, Mohammadi J, Shahsavarani H, Shokrgozar MA, Alipour A. Efficient mineralization and osteogenic gene overexpressions of mesenchymal stem cells on decellularized spinach leaf scaffold. *Gene* 2020; **757**: 144852 [PMID: 32599019 DOI: 10.1016/j.gene.2020.144852]

62 **Bae YK**, Kim GH, Kwon JH, Kim M, Choi SJ, Oh W, Um S, Jin HJ. Primary Cilia Mediate Wnt5a/β-catenin Signaling to Regulate Adipogenic Differentiation of Human Umbilical Cord Blood-Derived Mesenchymal Stem Cells Following Calcium Induction. *Tissue Eng Regen Med* 2020; **17**: 193-202 [PMID: 32008170 DOI: 10.1007/s13770-019-00237-4]

63 **Karam M**, Younis I, Elareer NR, Nasser S, Abdelalim EM. Scalable Generation of Mesenchymal Stem Cells and Adipocytes from Human Pluripotent Stem Cells. *Cells* 2020; **9** [PMID: 32183164 DOI: 10.3390/cells9030710]

64 **Lorthongpanich C**, Thumanu K, Tangkiettrakul K, Jiamvoraphong N, Laowtammathron C, Damkham N, U-Pratya Y, Issaragrisil S. YAP as a key regulator of adipo-osteogenic differentiation in human MSCs. *Stem Cell Res Ther* 2019; **10**: 402 [PMID: 31852542 DOI: 10.1186/s13287-019-1494-4]

65 **Hu L**, Yin C, Zhao F, Ali A, Ma J, Qian A. Mesenchymal Stem Cells: Cell Fate Decision to Osteoblast or Adipocyte and Application in Osteoporosis Treatment. *Int J Mol Sci* 2018; **19** [PMID: 29370110 DOI: 10.3390/ijms19020360]

66 **Meyer MB**, Benkusky NA, Sen B, Rubin J, Pike JW. Epigenetic Plasticity Drives Adipogenic and Osteogenic Differentiation of Marrow-derived Mesenchymal Stem Cells. *J Biol Chem* 2016; **291**: 17829-17847 [PMID: 27402842 DOI: 10.1074/jbc.M116.736538]

Published by **Baishideng Publishing Group Inc**
7041 Koll Center Parkway, Suite 160, Pleasanton, CA 94566, USA
Telephone: +1-925-3991568
E-mail: bpgoffice@wjgnet.com
Help Desk: https://www.f6publishing.com/helpdesk
https://www.wjgnet.com