

ORIGINAL ARTICLE

Cord lining progenitor cells: potential *in vitro* adipogenesis model

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Objective: To investigate the effect of glucose and insulin concentrations on differentiation of umbilical cord lining progenitor cells to adipocyte-like cells (ALCs).

Methods: Cord lining mesenchymal cells (CLMCs) were isolated from the explant of human umbilical cord amniotic membrane. CLMCs were subjected to differentiation under various culture conditions for 20 days. Lipid droplets were confirmed with Oil Red O staining. Gene expressions of *adipsin* and *peroxisome proliferator-activated receptor gamma* (*PPAR* γ) were analyzed using reverse transcription-PCR. Leptin and adiponectin secretions were detected using enzyme-linked immunosorbent assay kit.

Results: CLMCs became irregular, cuboidal-shaped cells that resemble adipocytes, and Oil Red O staining showed the presence of lipid droplets. The gene expressions of *PPAR* γ and *adipsin* were upregulated. Leptin and adiponectin secretions by naive CLMCs were below the limits of detection. Matured ALCs cultured in low-glucose medium significantly secreted leptin and adiponectin, whereas those in high-glucose medium significantly secreted only leptin. Insulin concentration affects leptin but not adiponectin secretion.

Conclusions: Under different culture conditions, CLMCs can differentiate into ALCs that resemble adipocytes in either normal-weight or obese individuals. Hence, these ALCs have the potential to be used as an *in vitro* model to study adipogenesis and obesity, and possibly as a drug discovery model for metabolic disorders.

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Introduction

Research in the field of stem cells has progressed by leaps and bounds in the past decade since the successful establishment of human stem cell lines.¹ There has been emerging evidence that these pluripotent cells have promising applications for regenerative medicine and cell therapies.^{2,3} Unfortunately,

the ethical concerns and controversies surrounding the usage of embryonic stem cells have been a major road block to its research and clinical applications. As such, multipotent mesenchymal progenitor cells are an attractive alternative. Although at a lower level of plasticity compared with embryonic stem cells, mesenchymal progenitor cells are sufficiently versatile for differentiation into certain cell lineages, providing a clinically safer alternative and an ethically acceptable option.⁴

Besides the widely used bone marrow, many mesenchymal stem cell niches have been identified, which includes adipose tissues,⁵ trabecular bone,⁶ amniotic fluid⁷ and Wharton's jelly.^{8,9} It has been found that mesenchymal stem cells can be isolated from the human umbilical cord in abundance.¹⁰ The umbilical cord mesenchymal stem cells have hematopoiesis-supportive function and the ability to differentiate in a similar manner as bone marrow-derived mesenchymal stem cells into osteogenic, chondrogenic, adipogenic and myogenic cells *in vitro*. The umbilical cord lining cells have tremendous advantages over other sources

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of progenitor stem cells. It is irrefutable that there will be an unlimited supply of umbilical cord lining cells as the birth of every baby is accompanied by an umbilical cord. Moreover, the umbilical cord is usually discarded as biowaste after delivery of the baby, and there is no mortality involved when the umbilical cord is harvested. Hence, there will be less ethical hassle with regard to the use of umbilical cord lining cells.

The escalating worldwide problem of obesity warrants a deeper understanding of adipogenesis and the imperative search for management of the disorder. The use of human umbilical cord lining mesenchymal cells (CLMCs) as an *in vitro* adipogenesis study model has not been reported to date. A recent improved method of isolating progenitor cells from the human umbilical cord has been patented by Phan.¹¹ This method of cell cultivation by tissue explants and isolation by medium selection is far more reliable and easier to perform to obtain two separate populations of mesenchymal and epithelial cells. This allows separate specific studies of epithelial and mesenchymal progenitor cells and hence widens the possibilities of cell-based therapy.

In this study, we investigated the effects of glucose and insulin concentrations on adipogenesis of CLMCs isolated by the tissue explant method. We illustrated that under different culture conditions, differentiation can be channeled toward an *in vitro* model that resembles adipocytes of a healthy, normal-weight or obese individual.

Materials and methods

Materials

Low-glucose Dulbecco's modified Eagle's medium, high-glucose DMEM, Ham-F12 medium, CMRL-1066 medium and antibiotic-antimycotic solution were purchased from Gibco (Carlsbad, CA, USA). DMEM was purchased from the National University Medical Institute, Singapore, and fetal bovine serum was from Hyclone, Thermo Scientific (Waltham, MA, USA). Transforming growth factor β -1, basic fibroblast growth factor, insulin-like growth factor and platelet-derived growth factor were from R&D Systems Inc (Minneapolis, MN, USA). CLMCs from three different umbilical cords were randomly selected from the specimen bank of CellResearch Corporation, Singapore, Singapore. The RNA extraction RNeasy Mini kit was purchased from Qiagen (Hilden, Germany), whereas the DNase I was from Fermentas (Burlington, Ontario, Canada). Oligonucleotide and $10 \times$ TAE buffer was from 1st Base, Singapore, Singapore. All primers, ReactionReady Human GAPD internal normalizer and ReactionReady Hotstart 'Sweet' PCR Master mix were purchased from SABiosciences (Frederick, MD, USA). The SuperScript III Reverse Transcriptase and Ultrapure agarose powder were from Invitrogen (Carlsbad, CA, USA). Gel Red was from Biotium Inc (Hayward, CA, USA). Loading dye and 100-bp DNA ladder were from Promega (Madison, WI, USA). Leptin and adiponectin enzyme-linked immunosorbent

assay (ELISA) kits were from RayBiotech Inc. (Norcross, GA, USA) and Amicon Ultra-15 10K centrifugal filter device was purchased from Millipore (Billerica, MA, USA). Pierce bovine serum albumin (2 mg ml^{-1}) was from Thermo Scientific, and protein assay dye reagent concentrate was from Bio-Rad (Hercules, CA, USA). Besides, β -mercaptoethanol and formaldehyde were from ICN Biomedical Inc. (Aurora, OH, USA), and ethanol was from Fisher Scientific (Hanover Park, IL, USA); all other chemical reagents were purchased from Sigma-Aldrich (St Louis, MO, USA), unless otherwise stated.

CLMCs database

Human umbilical cords were collected by CellResearch Corporation, Singapore with informed consent from the mothers after normal deliveries of healthy, full-term babies. All cells were isolated by the staff of CellResearch Corporation as described previously (international publication number WO 2006/019357 A1). Briefly, Wharton's jelly and blood vessels were separated from the umbilical cord amniotic membrane by dissection. The isolated amniotic membrane was cut into small pieces and explanted onto tissue culture dishes. Using the proprietary medium which contains a mixture of DMEM, Ham-F12 and CMRL-1066 media supplemented with human albumin, transforming growth factor β -1, platelet-derived growth factor, basic fibroblast growth factor, like growth factor and 2% fetal bovine serum, the primary cell line of mesenchymal cells was allowed to grow and proliferate. CLMCs used in the experiment were at either passage 3 or 4.

Adipogenesis

Cells were plated at a density of 5000 cells per cm^2 and cultured at 37°C in an atmosphere of 95% air and 5% carbon dioxide. Upon reaching confluency at passage 3 or 4, CLMCs were divided into different treatment groups as shown in Table 1. The groups of cells were incubated in the respective basal medium and insulin concentrations in the presence of adipogenesis induction agents (0.5 mM 3-isobutyl-1-methylxanthine, $200 \mu\text{M}$ indomethacin and $1 \mu\text{M}$ dexamethasone) for 6 days. This was followed by maintenance medium

Table 1 Medium content of various treatment groups

Group symbol	Basal medium	Insulin concentration ($\mu\text{g ml}^{-1}$)
N (Control)	DMEM	0
LGLI	LG-DMEM	10
LGHI	LG-DMEM	50
HGLI	HG-DMEM	10
HGHI	HG-DMEM	50

Abbreviations: FBS, fetal bovine serum; HG-DMEM; LG-DMEM, high-glucose Dulbecco's modified Eagle's medium; low-glucose Dulbecco's modified Eagle's medium. Besides group N, induction medium for all other groups also contain adipogenic agents (0.5 mM 3-isobutyl-1-methylxanthine, $200 \mu\text{M}$ indomethacin and $1 \mu\text{M}$ dexamethasone), 10% FBS and 1% antibiotics-antimycotic. Maintenance medium contain all respective components except adipogenic agents. LG-DMEM: 1 g l^{-1} D-glucose, HG-DMEM: 4.5 g l^{-1} D-glucose.

containing only the respective basal medium and insulin concentrations for 2 days. After two induction-maintenance cycles, the cells were incubated in the induction medium for another 4 days. Group N was incubated in DMEM throughout the 20 days of experiment. All five groups were supplemented with 10% v/v fetal bovine serum and 1% v/v antibiotics-antimycotic solution. The media were refreshed every 2–3 days.

Oil red O staining

Oil Red O staining was performed on days 3, 10 and 20 of differentiation to determine the accumulation of lipid droplets over time. Briefly, the cells were fixed in 4% v/v paraformaldehyde at room temperature for 1 h, and followed by staining with freshly prepared Oil Red O (in 60% v/v isopropanol) solution for 30 min. Excess stain was removed by washing with 70% v/v ethanol. Hematoxylin was used as nuclear counterstain. Cell staining was visualized under light microscopy.

Reverse transcription-PCR

Total RNA was extracted using the kit according to the manufacturer's instructions. The amount of RNA obtained was measured using a NanoDrop ND-1000 spectrometer (NanoDrop, Wilmington, DE, USA). Before synthesis of first-strand complementary DNA, RNA samples were treated with DNase I to eliminate genomic DNA contamination. Primers specific for peroxisome proliferator-activated receptor gamma (*PPAR*) γ and *adipsin* were used to synthesize complementary DNA from 1 μ g of total RNA. The reaction mixture was incubated initially at 95 °C for 10 min to activate the DNA polymerase, followed by thermal cycling at 95 °C, 15 s; 55 °C, 30 s; 72 °C, 30 s for 35 cycles. An additional 10 min of incubation at 72 °C was carried out after completion of the last cycle. The PCR end products were electrophoresed on 2% w/v agarose gel containing Gel Red in 1 $\times$ TAE buffer and visualized under ultraviolet light. Reverse transcription-PCR was performed in triplicates for each patient sample. Band-intensity signals of the PCR products were quantified using Gel-Pro Analyzer 4.5 by Media Cybernetics, Inc. (Silver Spring, MD, USA). Band intensities were calculated as the gene of interest-to-GAPD signal intensity ratio. The ratios obtained were analyzed using one-way analysis of variance (ANOVA) with Tukey's Honestly Significant Difference (HSD) *post hoc* comparisons.

ELISA

The media for each of the five adipogenesis treatment groups were collected at day 20 to assay for leptin and adiponectin secretion using the ELISA kits according to the manufacturer's instructions. The media were separately concentrated $\sim 80\times$ using the Amicon Ultra-15 10k centrifugal filter device from Millipore (Carrigtwohill, County Cork, Ireland). Total protein concentration was quantified by Bradford

protein assay before sample loading to ensure that equal amount of total protein was loaded into each ELISA well. The intensity of color change was measured at 450 nm using Tecan Infinite 200 series microplate reader (Tecan, Grödig, Austria). Data collected were analyzed using one-way ANOVA with Tukey's HSD *post hoc* comparisons, and two-way ANOVA.

Results

Cord lining mesenchymal cells

CLMCs are readily available from the umbilical cord lining, showed high proliferation activity and are easily maintained in the proprietary medium. They have spindle-like, fibroblastic morphology as illustrated in Figure 1. Previous data have shown that they are multipotent progenitor cells.^{11,12}

Morphology and Oil Red O staining

The morphology of CLMCs that were subjected to differentiation was observed to change from spindle-like fibroblastic cells at day 0 (Figure 1) to irregular, cuboidal-shaped cells that were adipocytes-like at day 20 (Figure 2). The intensity of Oil Red O staining in all four treatment groups gradually increased from day 1 to day 20, indicating accumulation of lipid droplets. Within the low-glucose treatment groups, higher insulin concentration resulted in more cells with lipid accumulation. The same phenomenon was observed for the high-glucose treatment groups. The sizes and accumulation rates of lipid droplets between low-glucose high-insulin and high-glucose low-insulin groups were not significantly different. Among the four treatment groups, it was observed that the rate of lipid droplets accumulation in cells was the slowest in the low-glucose low-insulin group and fastest in the high-glucose high-

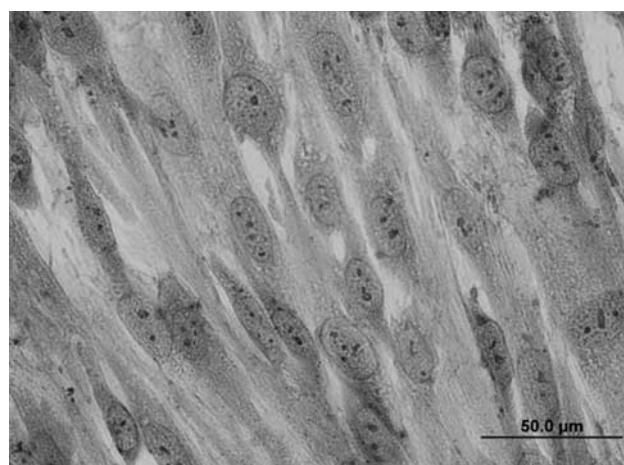


Figure 1 Naive cord lining mesenchymal cells.

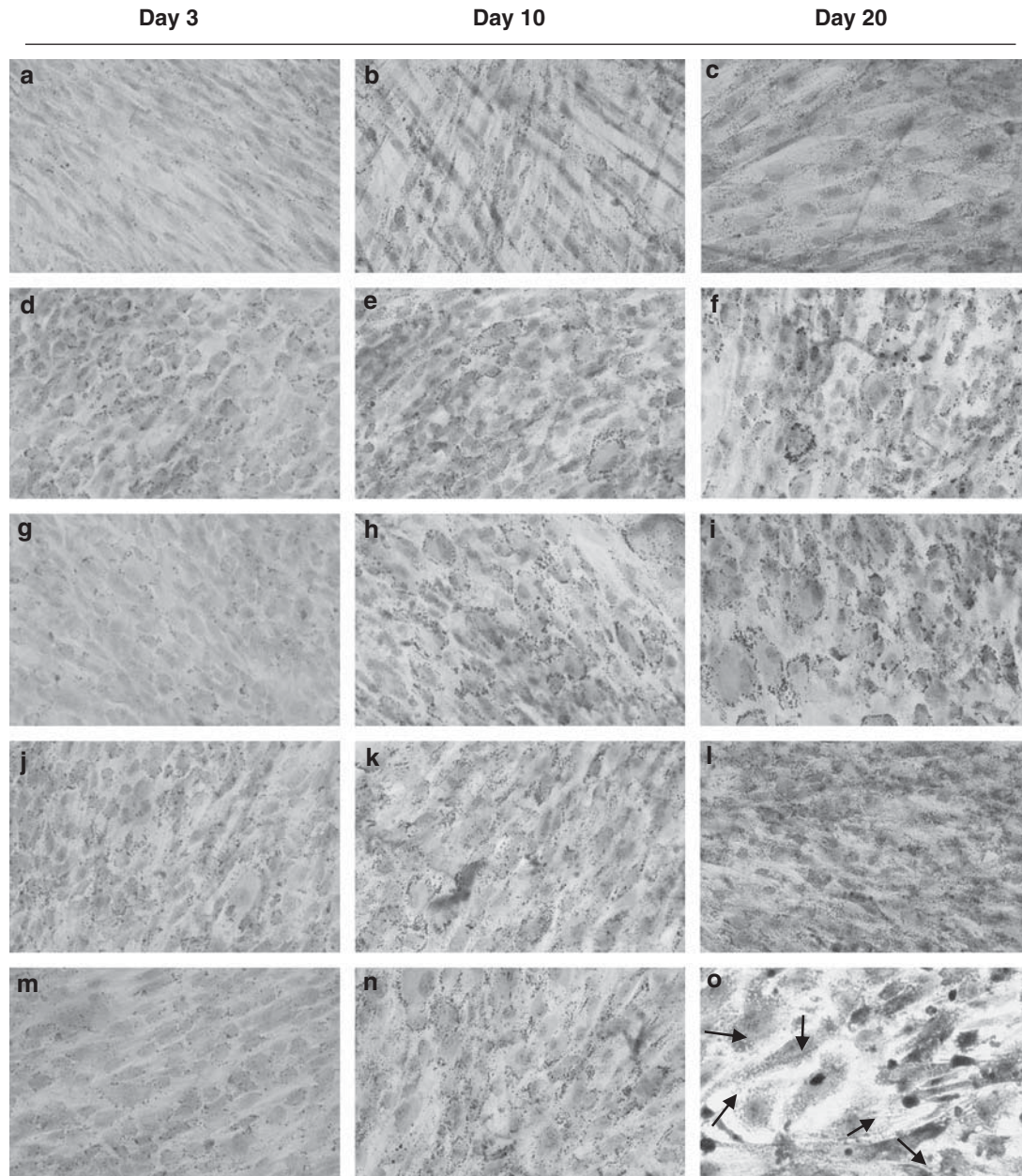


Figure 2 Cell morphology and Oil Red O staining of adipogenesis. (a–c) Control group N, (d–f) low-glucose low-insulin, (g–i) low-glucose high-insulin, j–l: high-glucose low-insulin, m–o: high-glucose high-insulin. At day 20, highest intensity of Oil Red O stain for lipids was seen in high-glucose high-insulin group as indicated by arrows. Pictures were taken under bright field setting.

insulin group. In addition, lipid droplets of cells cultured in the low-glucose low-insulin adipogenic medium were generally smaller in size than those in the high-glucose high-insulin adipogenic medium. At day 20, the high-glucose high-insulin group has the greatest intensity of Oil Red O staining. Tiny intracytoplasmic lipid droplets were observed in the control group. These lipid droplets in naive CLMCs are different from those observed in adipogenesis treatment groups, as the droplets were uniformly minute in size without increasing in amount or size over the 20 days.

On the other hand, lipid droplets in differentiation-induced groups were much bigger than those of the control group.

Reverse transcription-PCR

Adipsin. There was a gradual upregulation in the gene expression of *adipsin* for all differentiation-induced groups (Figure 3). Statistical analysis of band-intensity signals at day 20 by one-way ANOVA showed significant difference between control and induced groups, but there was no

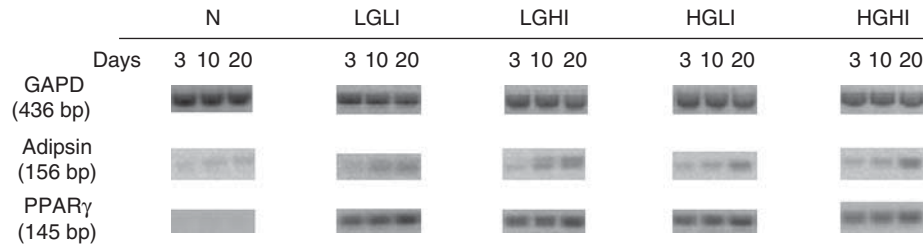


Figure 3 Gel electrophoresis of RT-PCR products.

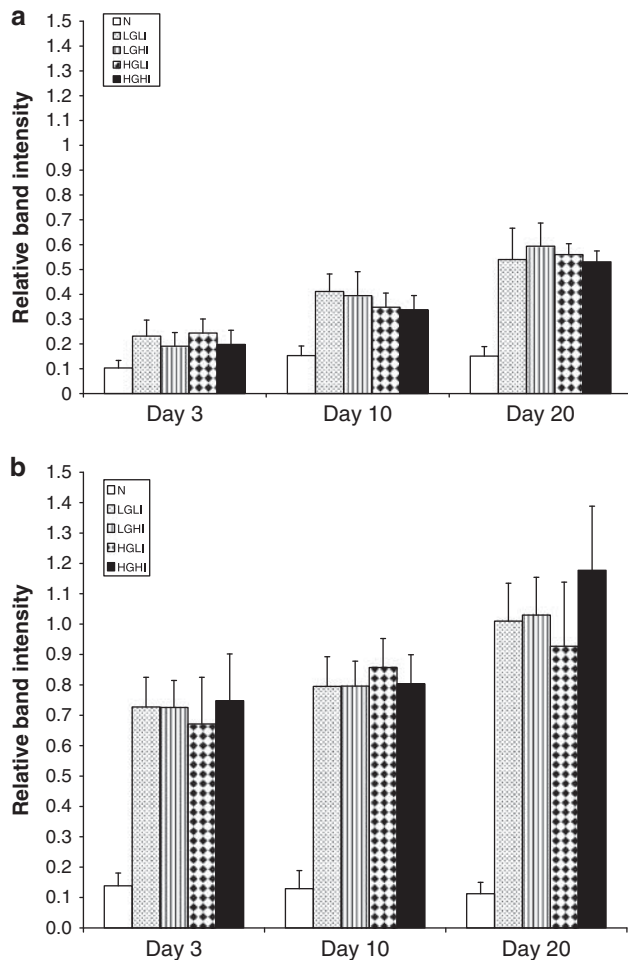


Figure 4 Relative band intensity of gene expression. After 20 days of differentiation, one-way ANOVA showed that there is a significant increase in the level of adipsin (a) and PPARγ (b) expression in all differentiation-induced groups as compared to control group N. Tukey's pairwise comparison showed no significant difference in the level of gene expression among differentiation-induced groups. Values are mean $\pm$ s.e.m.

significant difference in gene expression intensity among all differentiation-induced groups (Figure 4).

PPARγ. There was an obvious upregulation in the gene expression of *PPARγ* for all differentiation-induced groups

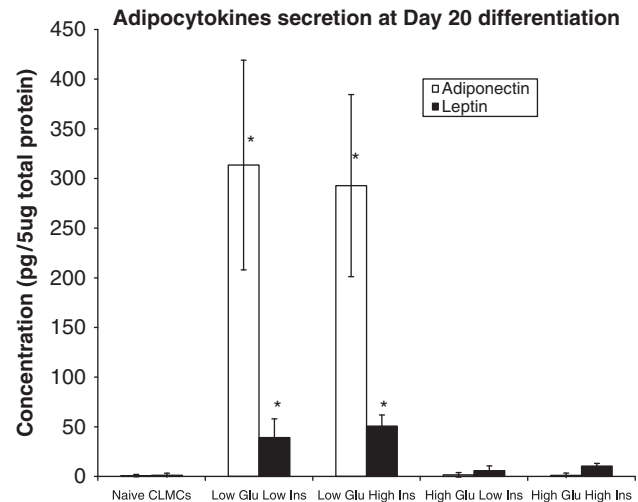


Figure 5 Adipocytokines secretion by ELISA at day 20. Limits of detection for adiponectin and leptin by the ELISA kit are 10 pg/ml and 6 pg/ml, respectively. Adiponectin: one-way ANOVA, Tukey's test showed significant difference between low-glucose adipogenic and naive CLMCs group ($P < 0.001$). Two-way ANOVA did not show any interaction between glucose and insulin ($P > 0.05$). Leptin: one-way ANOVA, Tukey's test showed significant difference between low glucose adipogenic and naive CLMCs group ($P < 0.001$). Two-way ANOVA showed that both insulin ($P < 0.05$) and glucose ($P < 0.001$) concentrations affect the amount of leptin secreted, but the two factors do not have any interactions ($P > 0.05$). Amount of adiponectin secretion in naive CLMCs, high-glucose low-insulin and high-glucose high-insulin groups was below 10 pg/ml⁻¹. Amount of leptin secretion in naive CLMCs and high glucose low insulin groups was below 6 pg/ml⁻¹. Values are mean $\pm$ s.e.m. *Significant difference.

(Figure 3). No gene expression was observed in the control group. Statistical analysis of band-intensity signals at day 20 by one-way ANOVA showed significant difference between control and induced groups, but there was no significant difference in the gene expression intensity among all differentiation-induced groups (Figure 4). The primer sequence used in this study recognized complementary DNA common to $\gamma 1$ and $\gamma 2$ isoforms.

ELISA

Leptin. The limit of detection for leptin secretion by the assay kit is 6 pg/ml⁻¹. The amount of secretion by naive CLMCs at day 20 was below the recommended limits of detection (Figure 5). Data analysis by one-way ANOVA

showed that there was no significant difference in leptin secretion between cells incubated in high-glucose medium and those of the control group. However, the amount of leptin secreted by matured adipocyte-like cells (ALCs) incubated in low-glucose medium groups was significantly more than those in the control group ($P < 0.001$). A further data analysis by two-way ANOVA on the effect of glucose and insulin concentrations and potential interactions between glucose and insulin showed that their concentrations significantly influenced the secretion of leptin ($P < 0.001$ and $P < 0.05$, respectively). There was no significant interaction between the two factors ($P > 0.05$).

Adiponectin. The limit of detection for adiponectin by the assay kit is 10 pg ml^{-1} . Although there was faint detection of adiponectin secretion by naive CLMCs and ALCs incubated in high-glucose medium at day 20, the amounts detected were below the recommended limits of detection (Figure 5). Data analysis by one-way ANOVA showed that different concentrations of glucose and insulin have a significant effect on the amount of adiponectin secreted. Tukey's test identified significant differences between the cells in the control group and those in low-glucose groups ($P < 0.001$). A further data analysis by two-way ANOVA showed that the concentration of glucose has significant effect ($P < 0.001$), whereas the concentration of insulin has no significant effect on the amount of adiponectin secretion ($P > 0.05$). There was no significant interaction between the two factors ($P > 0.05$).

Discussion

The physical and psychological impediment of obesity, coupled with social stigma is driving many individuals to seek ways to prevent or shed their excess weight. Obesity is a risk factor for increased morbidity and mortality rates of other conditions, such as diabetes, arthritis and cardiovascular diseases. It also causes abnormalities in the endocrine system and consequently an adverse bearing on reproduction. Undoubtedly, adipogenesis and obesity have been extensively studied in animal models, such as the *ob/ob* or *tubby* mouse model, or cell lines, such as the 3T3-L1 or F442A murine cell lines. Nonetheless, there will be genetic differences between animals and humans, which may be crucial to the study of obesity in humans. In addition, the use of human pre-adipocytes from the adipose tissue is not ideal. These cells, already committed to adipogenic lineages, have reduced proliferative ability and unpredictable variability based on different donors and anatomical sites.^{13,14}

The use of human bone marrow mesenchymal progenitor cells to study adipogenesis is well underway. These precursor cell types allow insights into biochemical pathways leading to adiposity and identification of crucial signaling molecules or pathways that may control the development of obesity and thus the discovery of therapeutic drugs for the

disease.^{15–17} However, invasive surgery is required to harvest bone marrow progenitor cells, causing undue stress and potential risk to the donor. In addition, Stolzinger¹⁸ reported that there was a reduced ability to maintain mesenchymal tissue homeostasis in aged mammals as a result of a decline in progenitor cell numbers and functionality due to the accumulation of oxidative damage. This translates to difficulty in finding enough donors to obtain sufficient progenitor cells of good quality for research and clinical applications. Human umbilical cord lining progenitor cells would serve as an alternative *in vitro* study model. All the umbilical cords collected are consistently young and healthy at an average gestational age of 40 weeks and the supply is bountiful.

In this study, we showed that mesenchymal progenitor cells can be easily harvested and maintained from the human umbilical cord lining using the tissue explant method. The tiny intraplasmic lipid droplets seen in naive CLMCs by Oil Red O staining are not unexpected, as CLMC is a primary cell line. Furthermore, the morphology of naive CLMCs remains spindle-like and fibroblastic. This is a stark contrast to the irregular and cuboidal shape of adipocytes. Therefore, it can be construed that CLMCs do not undergo adipogenesis in the absence of differentiation inducers. In the presence of adipogenic induction agents, CLMCs were stimulated to differentiate into ALCs as seen at day 20. All differentiation-treated groups were significantly stained by Oil Red O as compared with the control group and have cellular morphology similar to typical adipocytes. It was observed that higher concentration of glucose and insulin resulted in faster and more accumulation of lipid. Therefore, more intense Oil Red O staining was seen in the high-glucose high-insulin group, indicating that more lipid accumulated within the cells.

Adipogenesis in CLMCs was also confirmed at the genetic level, at which there is an obvious upregulation of the *adipsin* and *PPAR γ* gene expression in all differentiation-treatment groups. *Adipsin* is an immune system-related serine protease found to be identical to complement factor D.¹⁹ It is synthesized predominantly in adipose tissues and secreted into the blood stream. It was noted to be downregulated in several, but not all, models of obesity. Johnson *et al.*²⁰ reported a reduced *adipsin* mRNA and protein in genetically obese Zucker rats, and Lowell *et al.*²¹ also reported a decreased *adipsin* expression early in the development of obesity in *ob/ob* obese mouse model. However, in studies involving humans, *adipsin* was found to be higher with increased body mass.^{22,23} The upregulation of *adipsin* gene expression during adipogenesis in this study correlates well with similar observation reported by other research groups.^{15,24} *PPAR γ* is the most adipose specific of the *PPAR* subfamily of nuclear hormone receptors, and exists as two isoforms: *PPAR γ_1* and *PPAR γ_2* . It was shown that *PPAR γ* regulates the development of adipocyte lineage because its ectopic expression triggers adipogenesis in fibroblast and muscle cells.²⁵ The increased expression of

the *PPAR γ* gene observed in this study is evident that CLMCs had undergone adipogenesis. This is consistent with observations made by other groups dealing with mesenchymal cells.^{15,17,26} It is expected that there is no *PPAR γ* gene expression in naive CLMCs, as this gene is induced only before transcriptional activation of most adipocyte genes.²⁷ There is no significant difference in the intensity of gene expression among the four differentiation-treatment groups of different glucose and insulin concentrations. This suggests that together with other adipogenic-inducing agents, glucose concentration of 1 mg ml⁻¹ and insulin concentration of 10 μ g ml⁻¹ was sufficient to initiate adipogenesis of CLMCs. Increasing concentration of both factors did not affect the extent of initiating differentiation.

Mature adipocytes are known to secrete leptin and adiponectin.²⁷ Leptin, a 16-kDa *ob* gene product, is one of the most important adipose-derived hormones. It has a key role in regulating energy intake and expenditure, including appetite and metabolism.²⁸ The feedback regulatory loop acts through hypothalamic receptors to inhibit feeding and increase thermogenesis, resulting in a decrease in body weight. Serum leptin level correlates with fat mass, increases with obesity and is significantly higher in women than men even after adjustment for fat mass.^{29,30} Adiponectin, a 30-kDa protein has been shown to have a role in the regulation of glucose and lipid homeostasis and a paradoxical decrease in obesity and type 2 diabetes.³¹⁻³³ It is the most abundant hormone secreted by adipose tissue, and has inverse relationship with leptin. Its plasma concentrations are ≥ 1000 -fold higher than that of leptin, ranging from 1.9 to 17.0 mg ml⁻¹ in healthy volunteers.^{32,34,35} Körner *et al.* demonstrated in human adipocyte cell culture that adiponectin synthesis and secretion occurred specifically in mature adipocytes. Therefore, adiponectin could serve as a distinctive marker of adipocyte differentiation.³⁶

In our study, differentiated CLMCs after 20 days of incubation in low-glucose adipogenic medium secreted more adiponectin (low glucose low insulin: 313.6 $\pm$ 105.6 pg/5 μ g total protein, low glucose high insulin: 292.8 $\pm$ 91.2 pg/5 μ g total protein) and leptin (low glucose low insulin: 39.1 $\pm$ 18.9 pg/5 μ g total protein, low glucose high insulin: 50.6 $\pm$ 11.3 pg/5 μ g total protein) as compared with naive CLMCs (adiponectin: 0.9 $\pm$ 1.2 pg/5 μ g total protein, leptin: 1.2 $\pm$ 2.0 pg/5 μ g total protein) at day 20. This indicated that CLMCs had differentiated into mature ALCs and mimics the physiological adipocytokine secretion paradigm in normal-weight adults, in whom much more amount of adiponectin is present than leptin in the plasma. On the other hand, the amount of adipocytokines secreted by ALCs incubated in high-glucose adipogenic medium decreased drastically by ~ 300 -fold for adiponectin and 5-fold for leptin. The decrease in adiponectin secretion is likened to the occurrence in obesity. According to studies by other research groups on obesity, the inverse relationship between adiponectin and leptin concentrations should produce a decrease in adiponectin level and an increase in leptin level.^{32,34,35}

The lack of reduction in food intake and increase in energy expenditure despite elevated leptin concentration in human obesity have been linked to leptin resistance.³⁷ One of the mechanisms believed to account for leptin resistance is a defect in the transporter system of leptin through the blood-brain barrier, thus leptin is less efficient in obese individuals.³⁷⁻³⁹ The deficient in feedback regulatory mechanism leads to compensatory increase in leptin secretion to sustain regulation of energy intake and expenditure. With due consideration that the huge difference in magnitude of adipocytokines secretion between low- and high-glucose treatments may render two-way ANOVA less sensitive in detecting statistical significance; supplementary individual *t*-test was carried out between each differentiation-treatment group and naive CLMCs. The *t*-test result for ALCs in low-glucose differentiation media showed significant differences for leptin and adiponectin secretion, which matched the result of two-way ANOVA. On the other hand, for comparison between naive CLMCs and ALCs in high-glucose differentiation media, the *t*-test showed no significant difference for adiponectin secretion but significant difference for leptin secretion. This *t*-test result is different from that of two-way ANOVA, which failed to detect the statistical difference for leptin secretion. Therefore, our *in vitro* study shows that ALCs incubated in high-glucose medium is similar to adipocytes in obese individuals, in whom there is very low adiponectin secretion and high leptin secretion. Given the previous findings that leptin concentration increases with fat mass^{29,30} and the close resemblance of our ALCs in high-glucose medium to adipocytes in obesity, leptin secretion was expected to be higher in high-glucose treatment groups than in low-glucose treatment groups. However, this was not observed. The development of *in vivo* obesity involves a complex interplay of numerous cellular signals and pathways that has yet to be fully elucidated. The influence of other biochemical signaling such as those from the hypothalamus, on the overall increase in leptin concentration in obesity is beyond the interest of this study.

The above results taken together showed that CLMCs can be induced to ALCs. Under low-glucose culture conditions, ALCs obtained are comparable with adipocytes of normal-weight individuals as it secretes more adiponectin than leptin. Whereas under high-glucose culture conditions, ALCs obtained is likened to adipocytes of obese individuals due to the extremely small amount of adiponectin secreted. In addition, higher concentration of insulin enhanced lipogenesis.

As the penchant to overindulge and revel in a sedentary lifestyle becomes more prevalent with growing affluence of developing countries, obesity and other related diseases are mounting health concerns. The exact molecular and cellular involvements in the development of obesity and related metabolic diseases remain to be elucidated. In our bid to unravel the mystery of pathogenesis and pursuit for therapeutic solutions, the human cord lining progenitor cells possess the potential to be a good alternative source of

cells for *in vitro* study of diseases and development of therapeutics. In the presence of different glucose and insulin adipogenic media, ALCs resembling those of normal-weight or obese individuals was obtained.

Conflict of interest

Dr Phan Toan Thang is the chief research scientist at the CellResearch Corporation, Singapore.

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