Mature adipocytes and perivascular adipose tissue stimulate vascular smooth muscle cell proliferation: effects of aging and obesity

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Barandier, Christine, Jean-Pierre Montani, and Zhihong Yang. Mature adipocytes and perivascular adipose tissue stimulate vascular smooth muscle cell proliferation: effects of aging and obesity. Am J Physiol Heart Circ Physiol 289: H1807–H1813, 2005. First published July 15, 2005; doi:10.1152/ajpheart.01259.2004.—Adipocytes and perivascular adipose tissue are emerging as regulators of vascular function. The effects of adipocytes and perivascular adipose tissue on human smooth muscle cell (SMC) proliferation were investigated. Conditioned medium was prepared from cultured premature and differentiated 3T3-L1 adipocytes and from periaortic adipose tissue from young (3 mo) and old (24 mo) Wistar-Kyoto (WKY) rats, lean and obese Zucker rats (3 mo), and WKY rats fed normal chow or a high-fat diet for 3 mo. Conditioned medium from differentiated (but not premature) adipocytes stimulated SMC proliferation, which was abolished by charcoal and proteinase K treatment but was resistant to heat, trypsin, or phospholipase B (to hydrolyze lysophosphatidic acid). Further experiments demonstrated that the growth factor(s) are hydrosoluble and present in the fraction of molecular mass >100 kDa. Moreover, conditioned medium from periaortic adipose tissue stimulated SMC proliferation, which was significantly enhanced in aged rats and in rats fed a high-fat diet but not in obese Zucker rats deficient in functional leptin receptors. In conclusion, mature adipocytes release hydrosoluble protein growth factor(s) with a molecular mass >100 kDa for SMCs. Perivascular adipose tissue stimulates SMC proliferation, which is enhanced in aged WKY and in high-fat, diet-induced obesity but not in leptin receptor-deficient obese Zucker rats. These adipocyte-derived growth factor(s) and the effect of perivascular adipose tissue may be involved in vascular disease associated with aging and obesity.

THE PATHOGENESIS of coronary artery disease and its complications is multifactorial, involving vasoconstriction, thromboembolism, and vascular smooth muscle cell (SMC) proliferation (30). Risk factors such as aging, obesity, diabetes mellitus, and dyslipidemia adversely regulate the disease process (2, 33). Recent studies (6, 9, 14, 17) that used coculture or conditioned medium from differentiated 3T3-L1 adipocytes and from periaortic adipose tissue released from perivascular fat tissue has been recently proposed (11). A novel yet to be identified vascular relaxing factor(s) released from perivascular fat tissue has been recently proposed (11), suggesting that perivascular adipose tissue, the function of which is usually ignored in vascular biology studies, may play a role in the modulation of vascular functions. Therefore, the present study was designed to investigate the direct effects of adipocytes and perivascular adipose tissue on SMC proliferation and the influence of aging and obesity.

MATERIALS AND METHODS

Materials. Bovine insulin, BSA, indomethacin, phospholipase B, and all other chemicals were purchased from Sigma (Buchs, Switzerland). Proteinase K was obtained from Roche (Rotkreuz, Switzerland); 3-isobutyl-1-methylxanthine and dexamethasone were obtained from Calbiochem (Lucerne, Switzerland); rabbit polyclonal anti-phospho-(Thr202/Tyr204) p42/p44MAPK and anti-p42/p44MAPK antibodies were purchased from Cell Signaling Technology (Allschwil, Switzerland); anti-rabbit IgG alkaline phosphatase conjugate and Western Blue stabilized substrate for alkaline phosphatase were obtained from Promega (Wallisellen, Switzerland); FCS and media for SMC culture were purchased from Bioconcept (Allschwil, Switzerland); and media for murine 3T3-L1 adipocytes (American Type Culture Collection and LGC Promochem, Molsheim, France) were obtained from GIBCO (Basel, Switzerland).

Adipocyte culture and differentiation. Murine 3T3-L1 preadipocytes were cultured and differentiated in DMEM media with 10% FCS as described (19).

Preparation of adipocyte-conditioned medium. Conditioned medium from preadipocytes and differentiated adipocytes was prepared as follows: confluent cells were washed and kept in serum-free DMEM-Ham’s F-12 medium with 0.2% BSA without any growth factors (110 μM medium/cm²) for 24 h. The conditioned medium was collected and centrifuged (13,000 rpm for 10 min at 4°C) to remove cell debris. To analyze whether prostaglandins are involved in the stimulation of SMC growth, the adipocytes were treated with the cyclooxygenase inhibitor indomethacin (1 μmol/l) during the 24-h conditioning. To characterize the chemical features of the growth factor(s) released by the adipocytes, the adipocyte-conditioned medium (ACCM) was treated with trypsin (2 μg/ml) at 37°C for 2 h and/or heated at 65°C for 10 min or treated with proteinase K (10 μg/ml, 37°C, 2 h) and then heated at 90°C for 30 min, a condition that is required to inactivate the enzyme. The effect of being heated at a temperature of 90°C on the biological activity of ACCM was also

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individually tested. In another series of experiments, ACCM was treated with activated charcoal (10%) for 1 h. The charcoal was then removed by centrifugation at 13,000 rpm for 10 min followed by filtration through a filter paper with a pore size of 0.22 μm. To estimate the molecular mass of the growth factor(s), trypsinized and heated ACCM was subjected to molecular size sieving with the use of an Amicon ultrafiltration device fitted with a membrane with a molecular mass cutoff at 100 kDa (Ultrafree-0.5 centrifugal filter device; Millipore). Both the concentrate and filtrate reconstituted to the initial volume with Ham’s F-12 culture medium were assayed for their biological activity. These experiments showed that the growth-promoting effect of ACCM was mainly present in the concentrate. The trypsinized, heated, and ultrafiltered concentrate was then subjected to saturated butanol to separate liposoluble and hydrosoluble fractions.

**SMC culture.** SMCs were cultured from human saphenous veins (passages 6 to 14) or from human aorta (passages 9 to 11) as previously described (39).

**SMC proliferation.** SMCs were distributed in 96-well plates at an initial density of $2 \times 10^3$ cells per well and allowed to attach overnight. The cells were rendered quiescent in serum-free DMEM with 0.2% BSA for 48 h (38, 39) and then incubated with ACCM at different dilutions for 48 h. Cell proliferation was measured with the Celltiter 96 Aqueous One Solution Proliferation Assay kit (Promega), according to the manufacturer’s instructions or by counting the cell number over 8 days. The cells were stimulated with conditioned medium (100 μ/l/ml) every other day. Each measurement was performed in duplicate.

**p42/p44 MAPK activation.** The quiescent SMCs were stimulated with the ACCM (100 μ/l/ml) for the indicated times, and p42/p44 MAPK activation (phosphorylation at Thr202/Tyr204) was measured as described (38). Protein concentration was determined with the use of a DC Protein Assay kit (Bio-Rad). Quantification of the signals was performed by National Institutes of Health Image 1.62 software.

**Animals and preparation of perivascular adipose tissue-conditioned medium.** Three young (3 mo old) and three old (24 mo old) male Wistar-Kyoto (WKY) rats and three obese Zucker fa/fa and three lean Zucker fa/+ rats (male, 3 mo old) were fed normal chow. All rats were purchased from Harlan. In addition, three male WKY rats (3 mo) were fed a high-fat diet (60% of calories from fat; Provimi Kliba, Kaiseraugst, Switzerland) for a period of 3 mo, and three age- and sex-matched WKY rats for a period of 3 mo, and three age- and sex-matched WKY rats were fed normal chow during the same time period to serve as controls. All animals were maintained according to the local rules of animal experimentation. The experimental protocols were approved by the Ethics Committee for Animal Experimentation of the Veterinary Service, Fribourg, Switzerland. The animals were anesthetized with pentobarbital sodium (50 mg/kg body wt ip) and euthanized. The aortas were removed. Perivascular adipose tissue was dissected along the whole aortas and weighed. Four-hundred milligrams of fat tissue from each animal were collected and conditioned at $37^\circ$C in 1 ml of serum-free DMEM-Ham’s F-12 medium with 0.2% BSA for 4 or 24 h. The adipose tissue-conditioned medium (ATCM) was centrifuged, frozen, and kept at $-80^\circ$C until use. To characterize the biological activities of ATCM, the medium was treated with trypsin or proteinase K and/or heated as described in Preparation of adipocyte-conditioned medium.

**Statistics.** Cell proliferation was expressed as the percentage of the corresponding values of quiescent cells when assayed by the proliferation kit or as the number of cells per milliliter. Data were given as means ± SE. In all experiments, $n$ equals the number of experiments. Statistical analysis was performed by one-way ANOVA Tukey-Kramer multiple comparisons test or unpaired Student’s $t$-test. Differences were considered statistically significant at values of $P < 0.05$.

**RESULTS**

ACCM stimulates SMC proliferation. Conditioned medium from differentiated murine 3T3-L1 adipocytes (2 to 100 μ/l/ml) stimulated human saphenous vein and aortic SMC proliferation in a concentration-dependent manner (Fig. 1, A and B, 206 ± 21% increase in the vein and 145 ± 9% increase in the aorta at the highest concentration used, i.e., 100 μ/l/ml; $n = 5–6$, $P < 0.01$). Note that the relatively weaker response of the aortic SMCs is due to the intrinsically weaker proliferative activity of the cell line in general compared with the venous SMCs, because a weaker proliferative response to 5% FCS was also observed in aortic SMCs (299 ± 22% increase) compared with venous SMCs (425 ± 29%; $n = 4$, $P < 0.05$). The higher proliferative activity of venous SMC allows a more precise analysis of the functions of adipocytes on SMC proliferation, the following experiments were performed in the venous SMCs. Our previous studies (28, 37, 38) showed a similar growth property between the human saphenous vein and coronary artery SMCs.

Interestingly, conditioned medium from premature or undifferentiated adipocytes had no effect on SMC proliferation in both the saphenous vein and aorta (Fig. 1, A and B; $n = 6$). The SMC growth-promoting effect of the conditioned medium from mature adipocytes was not affected by treatment of the cells with the cyclooxygenase inhibitor indomethacin (1 μmol/l) during the 24-h conditioning (data not shown). Treat-

![Graph A](http://example.com/fig1a.png)  ![Graph B](http://example.com/fig1b.png)  ![Graph C](http://example.com/fig1c.png)

**Fig. 1.** Adipocyte-conditioned medium (ACCM) stimulates vascular smooth muscle cell (SMC) proliferation. ACCM of differentiated (but not premature) 3T3-L1 adipocytes (24-h conditioning) concentration dependently stimulated SMC proliferation (48-h stimulation) either from human saphenous vein (A) or aorta (B). Growth-promoting effect of ACCM in venous SMC was abolished by charcoal treatment (C). $*P < 0.05$ and $**P < 0.01$ vs. control, i.e., cells not treated with ACCM; $§P < 0.01$ vs. cells treated with ACCM alone. $n$, number of experiments.
ment of ACCM with charcoal fully eliminated the growth-promoting effect (Fig. 1C).

Incubation of ACCM with phospholipase B (3 U/ml, 37°C) for 30 min to hydrolyze LPA released from adipocytes caused only a 33 ± 3% inhibition of SMC proliferation (P < 0.05, n = 14) but a 93 ± 12% inhibition of p42/p44MAPK activation stimulated by ACCM (100 μg/ml, 10 min, P < 0.05, n = 3). Furthermore, SMC proliferation stimulated by ACCM (100 μg/ml) at 48 h or the increase in cell number stimulated by ACCM (100 μg/ml) over 8 days remained unaffected when the conditioned medium was heated (65°C, 10 min) or trypsinized (2 μg/ml, 37°C, 2 h, n = 3–4; Fig. 2, A and B), although p42/p44MAPK activation by ACCM (100 μg/ml, 10 min) was inhibited by over 70% by the treatments (Fig. 2C).

Despite being resistant to trypsin, ACCM was sensitive to proteinase K treatment. The results in Fig. 2, A and B, show that heating ACCM at 65°C for 10 min had no effect on its growth-promoting activity. We further show that heating ACCM at a higher temperature, i.e., 90°C for 30 min (a condition that is required to inactivate proteinase K), only slightly reduced its growth-promoting effect (22% reduction, P < 0.05, Fig. 3), further confirming the heat-resistant feature of the growth factor(s) contained in the conditioned medium. This heat-resistant component of ACCM was, however, abolished by proteinase K treatment (10 μg/ml, 37°C, 2 h; Fig. 3).

Ultrafiltration of heated and trypsinized ACCM with a membrane with a molecular mass cutoff at 100 kDa showed that the growth-promoting effect was retained in the fraction that has a molecular mass >100 kDa (Fig. 4A). The result indicates that the active substance(s) released from cultured adipocytes have a molecular mass >100 kDa. Furthermore, lipid extraction of heated and trypsinized ACCM with butanol showed that the growth-stimulating activity of ACCM was retained in the hydrosoluble fraction but not in the liposoluble fraction (Fig. 4B).

Conditioned medium from perivascular adipose tissue stimulates SMC proliferation: effects of aging and obesity. The effect of perivascular adipose tissue on SMC growth and the impact of aging and obesity were further investigated. We showed that ATCM prepared from the periaortic fat of 3-mo-old WKY rats (4 h conditioning) also stimulated SMC proliferation (Fig. 5). In contrast to ACCM, the growth-stimulating effect of ATCM (100 μg/ml) was partly sensitive to heat either at 65°C for 10 min (P < 0.01, Fig. 5A) or 90°C for 30 min (P < 0.01, Fig. 5B). It is resistant to trypsin (Fig. 5A). Indeed, trypsin treatment of ATCM alone or in combination with heat did not affect the growth-promoting effect of ATCM (Fig. 5A). The growth-promoting activity of ATCM was, however, further reduced by proteinase K treatment (Fig. 5B). Note that a significant portion of the growth-promoting activity of ATCM was still retained after combined treatment of heat plus trypsin (41%, P < 0.01 vs. ATCM, Fig. 5A) or heat plus proteinase K (40%, P < 0.05 vs. ATCM, Fig. 5B).

To study aging- and obesity-dependent effects, young (3 mo) and old WKY rats (24 mo) and two obesity animal models, namely, Zucker fa/ fa rats deficient in functional leptin receptors and high-fat, diet-induced obese WKY rats were

Fig. 2. SMC proliferation stimulated by ACCM is heat and trypsin resistant. Neither cell proliferation (A) nor increase in cell number (B) stimulated by ACCM (100 μg/ml, 24-h conditioning) of mature adipocytes over 8 days was affected by treatment of ACCM by heating (65°C, 10 min) or trypsin (2 μg/ml, 37°C, 2 h), whereas p42/p44MAPK activation (phosphorylation at Thr202/Tyr204) stimulated by ACCM (100 μg/ml, 10 min) was strongly attenuated by treatments (C). *P < 0.05 and **P < 0.01 vs. control; $P < 0.05 and $$P < 0.01 vs. cells treated by ACCM alone.

Fig. 3. Treatment of ACCM with proteinase K (PK, 10 μg/ml, 37°C, 2 h) abolishes its growth-promoting effect. SMC growth-promoting effect of ACCM was only slightly reduced by heating at a higher temperature, i.e., 90°C for 30 min, a condition that is required to inactivate PK. Effect of ACCM was, however, further abolished by PK treatment. **P < 0.01 vs. control; #P < 0.05 and ##P < 0.01 vs. ACCM; $P < 0.05 vs. heated ACCM.
used. The body weights of old (24 mo) and high-fat diet-fed obese (6 mo) WKY rats and those of obese Zucker fa/fa rats (3 mo) were comparable and significantly higher compared with their respective controls (Table 1). Although the old WKY rats had a greater absolute amount of periaortic fat tissue than did the young rats, the ratio of periaortic fat to body weight, however, was comparable between the two groups (Table 1). Interestingly, the absolute amount of periaortic fat tissue and the ratio of periaortic fat to body weight were higher in the two obesity animal models than in the lean littermates (Table 1).

Perivascular ATCM (100 μl/ml) prepared from 4-h conditioning, concentration dependently stimulated SMC proliferation (Fig. 6A). This effect was significantly enhanced in aged and high-fat diet-fed obese WKY rats compared with the young and lean animals, respectively (Fig. 6A and B). In contrast, this effect of periaortic adipose tissue was, however, less pronounced in obese Zucker fa/fa rats compared with the lean littermates (Fig. 6C).

**DISCUSSION**

Recent research (22, 23, 26, 29, 31) indicates that adipocytes release hormones regulating vascular functions including SMC growth. In the present study, we showed that conditioned medium from differentiated mature adipocytes but not that from premature or undifferentiated adipocytes stimulated SMC proliferation, suggesting that a growth factor(s) is specifically produced from differentiated adipocytes. In line with our results, a recent study by Manabe et al. (20) demonstrated that mature primary adipocytes, but not preadipocytes, isolated from subcutaneous fat tissue of Wistar rats stimulate breast cancer cell proliferation. Our results, however, contrast with those of an early study (4) that showed no growth-promoting effect on rat and calf aortic SMC with conditioned medium from 3T3-F442A adipocytes. The discrepancy might be due to the different experimental conditions used, such as different adipocytes or SMC lines or different dilutions of the preparation of the conditioned medium.
The chemical nature of the SMC growth factor(s) released from mature adipocytes shown in our present study was further investigated. The involvement of prostaglandins can be excluded, because conditioned medium from mature adipocytes treated with the cyclooxygenase inhibitor indomethacin did not affect the growth-promoting effect. Indomethacin has also been shown to activate peroxisome proliferator-activated receptor-γ (PPAR-γ) in adipocytes (15). PPAR-γ activation reduces the release of free fatty acids and adipokines mediating insulin resistance such as TNF-α, leptin, and resistin but increases SMC growth inhibitor adiponectin production. PPAR-γ activation in SMCs has been reported to cause cell growth arrest (for details, see Ref. 21). As a consequence of PPAR-γ activation, the proliferation of SMCs stimulated by ACCM should be inhibited by indomethacin treatment. However, this is not the case, suggesting that those factors that are regulated by PPAR-γ could not be responsible for the growth-promoting effect of ACCM. The factor(s) can be adsorbed by charcoal, because charcoal fully eliminated the growth-promoting effect of the ACCM. It is known that adipocytes are able to produce estrogen, which promotes breast cancer cell growth (20, 35). However, estrogen cannot account for the SMC growth-promoting effect because estrogen does not stimulate but inhibits SMC proliferation as shown by our previous study (7). In addition, adipocytes also produce a significant amount of LPA, a lipid that has been shown to stimulate SMC growth (12). In our experiments, treatment of ACCM with phospholipase B, the enzyme that hydrolyzes LPA (10), only partially reduced the growth-stimulating effect (33% inhibition) but largely attenuated p42/p44MAPK activation by the conditioned medium (93% inhibition). These results suggest that LPA only partially contributes to the observed growth-stimulating effect.

The most important finding of our present study is that the growth-promoting effect of the conditioned medium from mature adipocytes is heat and trypsin resistant but proteinase K sensitive (Figs. 2 and 3), suggesting the proteinous nature of the growth factor(s). Further experiments with ultrafiltration demonstrated that the growth-promoting effect of the ACCM was retained in the fraction that has a molecular mass >100 kDa (Fig. 4A). Moreover, lipid extraction of heated and trypsinized ACCM showed that the growth-promoting effect is present in the hydrosoluble fraction (Fig. 4B), suggesting the water-soluble nature of the growth factor(s). The high molecular mass with the feature of trypsin and heat resistance might imply a protein with a globular structure. Note that p42/p44MAPK activation by the conditioned medium was markedly inhibited by trypsin or heating, implying that other factors that stimulate p42/p44MAPK were inactivated and that the heat- and trypsin-resistant growth factor(s) stimulate SMC proliferation mainly independently of p42/p44MAPK. Note also that heating, trypsin digestion, and phospholipase B treatment all independently reduced p42/p44MAPK activation by over 70%, which may be due to the synergistic effects of LPA and other growth factors (8).

Blood vessels are surrounded by adventitial perivascular fat tissue that is usually ignored by experiments investigating vascular functions. It is emerging that the perivascular adipose tissue may play a role in regulation of vascular functions as suggested most recently. Indeed, these studies (18, 36) showed that perivascular adipose tissue from rat aortas or mesenteric arteries produces a SMC relaxing factor(s). In the present study, we further demonstrated that periaortic adipose tissues also release growth factor(s) stimulating SMC proliferation (Fig. 5). The chemical features of the biological activity of ATCM were studied and compared with ACCM. The SMC

Table 1. Body weight and perivascular fat in aging and obesity

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<th>Wistar-Kyoto Rats</th>
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<td></td>
<td>Young</td>
<td>Old</td>
</tr>
<tr>
<td>Body weight, g</td>
<td>339 ± 12</td>
<td>469 ± 13*</td>
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<tr>
<td>Perivascular fat weight, g</td>
<td>0.4 ± 0.1</td>
<td>0.6 ± 0.1</td>
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<td>Perivascular fat-to-body weight ratio (× 10⁻³)</td>
<td>1.1 ± 0.1</td>
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Values are means ± SE; n = 3 rats per group. *P < 0.01 vs. young; ‡P < 0.01 and †P < 0.05 vs. lean.

Fig. 6. Effects of periaortic ATCM on SMC growth in aging and obesity. ATCM stimulated SMC proliferation, which was significantly enhanced in aged (A) and obese (B) Wistar-Kyoto (WKY) rats but decreased in obese Zucker fa/fa rats (C). *P < 0.05 and **P < 0.01 vs. control; §§P < 0.05 and §§§P < 0.01 young vs. old rats and lean vs. obese rats at same concentration of ATCM.
growth-promoting activity of ATCM was partly reduced by heat (Fig. 5). The remaining activity of ATCM after heating was resistant to trypsin but partly sensitive to proteinase K, suggesting that the proteinous component produced by cultured mature adipocytes is also present in ATCM. It is important to note that about 40% of the biological activity of ATCM is still observed after heating and proteinase K treatment (Fig. 5B), suggesting a nonproteinous component produced from perivascular tissue. It is not surprising to observe a more complex feature of ATCM than that of ACCM, because perivascular adipose tissue contains heterogeneous cell types such as endothelial cells, fibroblasts, and mesenchymal stromal cells. It remains for future studies to analyze the individual components released by the different cells in the perivascular adipose tissues under physiological and pathological conditions.

Nonetheless, the sum effect of perivascular adipose tissue on SMC growth was investigated in aging and obesity animal models. The results showed that the growth-promoting effect of periaortic adipose tissue was significantly enhanced in aged rats (Fig. 6A), suggesting that it might be involved in age-associated vascular intimal thickening or remodeling (16), although this effect appears modest under our in vitro experimental conditions. One could presume that during the chronic aging process, which is associated with increased absolute fat mass deposition around blood vessels (Table 1) and deterioration of vascular protective mechanisms such as endothelial functions (16), the growth-promoting effect of perivascular adipose tissue could become dominant and promote negative vascular remodeling and vascular diseases.

The impact of obesity on perivascular adipose tissue-induced SMC growth was studied in two obesity models. In genetically obese Zucker fa/fa rats deficient in functional leptin receptors, the growth-promoting effect of perivascular adipose tissue, to our surprise, was less pronounced (Fig. 6C). In contrast, in the high-fat diet-induced obese WKY rats, the SMC growth-promoting effect of periaortic fat was significantly increased compared with the control group (Fig. 6B). In both obesity models, the amount of periaortic fat is almost twice as much in the obese rats as in the lean rats, and the ratio of periaortic fat to body weight is higher in the obese rats than in the lean rats (i.e., perivascular fat mass increases proportionally more than body mass in the obesity model; see Table 1). It is presumable that the SMC growth-stimulating effect of perivascular adipose tissue might become even more important in obesity, in particular, when adipocyte-derived growth promoters such as leptin and TNF-α are increased and growth inhibitors such as adiponectin are decreased (13, 24).

The contrasting results between the two obesity models might be due to the fact that in obese Zucker fa/fa rats, there is no effect of leptin due to leptin receptor mutation. Leptin has been shown to be associated with an increase in cardiovascular risk (5). Genetically obese Zucker fa/fa rats are also relatively resistant to atherosclerosis and changes in vascular functions (1, 3, 34). Also, the production of the newly suggested relaxing factor(s) released from periaortic adipose tissue in Zucker fa/fa rats remains preserved as demonstrated recently (18). Interestingly, in high-fat diet-induced obese WKY rats, a more relevant obesity model, the SMC growth-stimulating effect of perivascular ATCM was significantly enhanced compared with the lean controls (Fig. 6B), implying a more pronounced release of SMC growth factors from perivascular adipose tissue of the obese rats.

In conclusion, our study suggests that mature adipocytes release a hydrosoluble protein growth factor(s) with a molecular mass >100 kDa for SMC. Perivascular adipose tissue stimulates SMC proliferation, which is enhanced in aged WKY and in high-fat diet-induced obesity. This adipocyte-derived growth factor(s) and the effect of perivascular adipose tissue may be involved in vascular disease associated with aging and obesity.

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ADIPOCYTES STIMULATE SMC GROWTH


