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Short Report

Increased fibrosis and angiogenesis in subcutaneous gluteal adipose tissue in nascent metabolic syndrome

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ABSTRACT

Aims. – Metabolic syndrome (MetS) is globally a common disorder that predisposes to both diabetes and cardiovascular disease (CVD). There is a paucity of data on fibrosis and angiogenesis in adipose tissue (AT) in patients with nascent MetS uncomplicated by diabetes or CVD. Hence, we assayed various indices of fibrosis and angiogenesis in subcutaneous AT (SAT).
Methods. – In both patients with MetS and matched controls, we determined fibrosis and the densities of CD31, VEGF and Angiopoietin (Angio) 2 and 1 by immunohistochemistry in gluteal SAT.
Results. – The fibrosis score was significantly increased in SAT of Met S. Also, both CD31 and VEGF densities were significantly increased. Surprisingly, Angio-2 was not increased and the ratio of Angio2:1 was decreased. Both indices of fibrosis and angiogenesis correlated with biomediators of inflammation.
Conclusions. – In conclusion, we report increased fibrosis and paradoxical increased angiogenesis in gluteal SAT and speculate that the increased angiogenesis is a protective mechanism in mitigating further adipose tissue dysregulation in this depot.

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Introduction

Metabolic syndrome (MetS), which affects 35% of US adults, is globally a very common disorder and confers an increased risk for diabetes and cardiovascular disease [1]. There is sparse data on adipose tissue (AT) dysfunction in nascent MetS without the confounding of diabetes or cardiovascular disease. Previously, we have reported a dysregulation of adipokines and increased macrophage density in AT including crown–like structures [2,3]. Given the scanty data on adipose tissue fibrosis and angiogenesis in MetS and the conflicting data especially with respect to adipose tissue angiogenesis in obesity [4–8], we examined indices of both fibrosis and angiogenesis in subcutaneous AT (SAT) from patients with MetS to gain further insights.

Research design and methods

Patients and methods

Subjects (aged 27–69 years) with MetS ($n = 20$) and healthy controls ($n = 15$) who had available adipose tissue and biochemical sample data were recruited from Sacramento County using procedures as described previously [2,3,9,10]. MetS was defined using the criteria of the NCEP ATP III [1]. Briefly, nascent MetS is defined as patients with MetS without diabetes (fasting plasma glucose < 125 mg/dL and $HbA_{1C} < 6.5\%$ (< 47.5 mmol/mol) or clinical CVD (coronary artery disease, peripheral arterial disease, cerebrovascular disease) as reported previously [2,3,9]. Control subjects had to have ≤ 2 features of MetS and not be on blood pressure medications. Other exclusion criteria for control subjects were fasting plasma glucose (> 100 mg/dL) and triglycerides (TGs) (> 200 mg/dL).

None of the subjects in both groups were on statins, angiotensin receptor blockers, fibrates, aspirin or PPAR (peroxisome proliferator activated receptor) gamma agonists or high dose antioxidant supplements. All subjects had a C-reactive protein (hsCRP) < 10 mg/L and normal white cell and had no acute or

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Table 1
Salient baseline characteristics.

Variable	Controls ($n = 15$)	MetS ($n = 20$)	P-value Controls vs. MetS
Sex, F/M (n)	14/1	15/5	0.21
Age (yrs)	47 ± 14	53 ± 10	0.12
Waist (cm)	94 ± 14	112 ± 16	0.002
BMI (kg/m^2)	31.3 ± 4.7	36.1 ± 6.7	0.02
BP-systolic (mmHg)	124 ± 12	126 ± 13	0.56
BP-diastolic (mmHg)	78 ± 7	80 ± 10	0.56
Glucose (mg/dL)	87 ± 8	102 ± 12	0.0002
Total cholesterol (mg/dL)	186 ± 30	188 ± 29	0.81
Triglycerides (mg/dL)	83 (60, 97)	123 (96, 160)	0.001
HDL cholesterol (mg/dL)	51 ± 13	42 ± 12	0.04
LDL cholesterol (mg/dL)	118 ± 24	121 ± 21	0.62
hsCRP (mg/L)	1.8 (0.4, 4.0)	4.0 (1.8, 6.0)	0.02
HOMA-IR	1.1 (1.07, 2.8)	3.3 (2.4, 5.1)	0.0003
FFA	0.44 ± 0.19	0.79 ± 0.12	0.0002
Plasma IL-1b (pg/mL)	482 (256, 844)	875 (797, 1187)	0.01
Plasma IL-6 (pg/mL)	874 (456, 1425)	1871 (1693, 2018)	0.0002
Plasma IL-8 (pg/mL)	625 (546, 746)	1142 (814, 1887)	0.02
Plasma chemerin (ng/mL)	253 ± 49	360 ± 60	0.0003
pp38/p38 MAPK	0.07 ± 0.03	0.23 ± 0.12	0.0002
sTNF-R1 (pg/mL)	856 ± 177	1227 ± 219	0.0005
Adipose tissue Angiopoietins density (rau/hpf)			
Angio 1	1.4 (0.9, 1.8)	3.1 (1.7, 5.1)	0.0002 0.0006*
Angio 2	1.1 (0.9, 1.6)	1.1 (0.8, 1.2)	0.66 0.78*

Results are presented as mean ± standard deviation or median (25th percentile, 75th percentile). Continuous variable comparisons between groups are made with two-sample t-tests; transformations were applied for skewed distributions. rau: relative arbitrary units; hpf: high power field.
* Adjusted for age and waist circumference.

chronic inflammatory diseases or recent infection. All other exclusion criteria including smoking, albuminuria > 30 mg/G creatinine, clinical cardiovascular diseases are detailed in previous publications [2,9].

Informed consent was obtained from participants in the study, which was approved by the institutional review board at the University of California Davis. After a 10-hour fast, blood was obtained for basic chemistries, hsCRP, and homeostasis model assessment (HOMA) calculation as described previously [2,9]. Plasma cytokines were assayed by multiplex ELISA as described previously [2,9]. Phospho-P38MAPKinase activity was assayed by a validated bioplex multiplex phospho-protein detection system [10].

Subcutaneous adipose tissue (SAT) biopsy was performed on all subjects as described previously [2], fixed in 10% buffered formalin after cleaning and processed for immunohistochemistry as described previously [11]. Briefly, sections were deparaffinized and boiled for 10 minutes in 10 mM sodium citrate buffer (pH 6.0), incubated with 10% normal horse serum followed by 60-minute incubation with primary antibodies or an appropriate isotype negative control. Sections were exposed to H_2O_2 for 5 min and then incubated with biotinylated anti-rat IgG (BD Biosciences Pharmingen, San Diego, CA) or anti-rabbit IgG (BD Biosciences Pharmingen, San Diego, CA) or anti-goat IgG respectively (Vector Laboratories Inc., Burlingame, CA). A Vector stain ABC kit (Vector Laboratories Inc., Burlingame, CA) was applied to the tissue followed by DAB solution (DAKO). The slides were counterstained with haematoxylin. Sirius red staining was performed for fibrosis. Quantification of immunostaining was assessed for all the images using ImageJ and expressed as relative absorbance units.

Also, we have previously reported on adipokines and other inflammatory biomediators in our patients with Met S [2,3,9].

Statistical analysis

Results are expressed as mean and standard deviation (SD) or as median and interquartile range. Log or square root transformations were applied to variables with skewed distributions prior to parametric analyses.

Comparisons between the control and MetS groups were made with two-sample t-tests and analysis of covariance to control for age and waist circumference (WC). Significance was defined as a P value < 0.05. The association of these indices with number of features of MetS and P for trend was derived using Jonckheere–Terpstra test. Combining the control and metabolic syndrome groups, Spearman rank correlation coefficients were computed to assess the association between adipose tissue CD31, VEGF, fibrosis and metabolic and other variables. Data were analyzed using SAS version 9.4 (SAS Institute, Cary, NC).

Results

As shown in Table 1, the cardio-metabolic features were significantly abnormal in patients with MetS as in our previous reports [2,9]. In addition, hsCRP, HOMA-IR, phospho-p38MAPkinase, and IL1, IL6, IL-8 chemerin and sTNFR-1 levels were significantly increased.

There was increase in the fibrosis score in SAT of patients with MetS compared to controls, even following adjustment for waist circumference (WC) as shown by the Sirius red staining (Fig. 1). With respect to angiogenesis, there was increased staining for both CD31 and VEGF in SAT following adjustment for WC. Whilst there was a significant increase in staining for Angiopoietin-1, there was no difference in Angiopoietin-2 staining resulting in a significant decrease in Angio2/Angio1 ratio in MetS. Representative staining is shown in Supplementary data, Fig. S1; see supplementary material associated with this article online. There were significant increases in CD31, VEGF and Fibrosis Score in SAT with increasing features of MetS ($P < 0.001$). Also, there was a significant increase in Angio-1 ($P = 0.03$) and a decrease in Angio-2/Angio-1 ratio with increasing features of MetS ($P = 0.04$).

Relevant correlations were undertaken with the above markers with a P value < 0.01 defined as significant. CD31 significantly

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