located outside this LD block (Fig. 1B), we judged *SCG3* to be a candidate susceptibility gene for obesity. *P* values of 39 SNPs located in block 2 and the adjacent blocks 1 and 3 are indicated in Fig. 1B. Among 40 genetic polymorphisms within the *SCG3* gene that we found and genotyped, 11 SNPs [SNP-2 (rs16964465), 5′ flanking −1203; SNP-5 (rs3809498), 5′ flanking −65; SNP-9 (rs16964476), intron 1 + 190; SNP-11 (ssj0011012), intron 1 + 478; SNP-12 (rs3214014), intron 1 + 605; SNP-16 (rs2305709), exon 4 + 351(I117I); SNP-17 (rs3816544), intron 4 + 127; SNP-20 (rs2305715), intron 5 + 677; SNP-26 (rs2305719), intron 6 + 2677; SNP-27 (ssj0011013), intron 8 + 25; SNP-29 (rs3765067), intron 9 + 52] were in almost complete linkage disequilibrium ($\Delta = 0.99–1.0$) with SNP-1 and also revealed significant associations with obesity (Fig. 1B). For example, the frequency of the subjects with the C/C genotype at SNP-2 was significantly lower in the obesity group than the control group (odds ratio 9.23; 95% CI 2.77–30.80, χ^2 19.2, $P = 0.0000067$) (Supplemental Table 1, published as supplemental data on The Endocrine Society's Journals Online Web site at http://jcem.endojournals.org). The remaining SNPs showed no significant association with obesity. SNPs in the 5′-flanking region are counted from the transcription initiation site. For SNPs in introns, nucleotide positions are counted from the first intronic nucleotide at the exon-intron junction and for SNPs in exon regions, from the first exonic nucleotide (transcription initiation site) according to sequence accession no. AC020892.7 and NM_013243.2.

Regulatory effect of SNPs on SCG3 expression

Three SNPs (SNP-1, SNP-2, SNP-5) were located in the 5′ flanking region and three SNPs (SNP-9, SNP-11, SNP-12) were located in intron 1 of SCG3 gene, regions that could putatively affect transcriptional activity. To examine whether these six SNPs would affect the transcriptional activity, we performed a luciferase assay using the neuroblastoma cell-line SH-SY5Y, which has previously been shown to express SCG3 (25). Between the major and minor alleles at each locus, only the clones containing SNP-2 or SNP-9 showed significant differences in transcriptional activity (Fig. 2A), and these differences were enhanced using the plasmids containing four concatenated copies of these DNA fragments, suggesting that SNP-2 and SNP-9 were able to affect the transcriptional activity of the SCG3 gene. SCG3 was also reported to be expressed in pancreatic β-cells (13); thus, we performed the same experiments using the hamster pancreatic β-cell line, HIT-T15 (Fig. 2A). We observed similar results, although the differences in the transcriptional activity between SNPs using HIT-T15 cells were smaller than those seen in the SH-SY5Y cells, probably due to the species difference.

To further investigate whether the regions containing each of these six SNPs can act as target binding sites of nuclear protein(s), we performed a gel-shift assay using SH-SY5Y cell extract and oligonucleotides corresponding to genomic sequences that included major or minor alleles of each of the six SNPs (SNP-1, SNP-2, SNP-5, SNP-9, SNP-11, and SNP-12). The band corresponding to the minor allele (C allele) of SNP-2 was more intense than that corresponding to the major allele (A allele) (Fig. 2B), indicating that some nuclear factor(s) has higher binding affinity to the minor allele. Although we observed shifted bands for the oligonucleotides corresponding to SNP-5 and SNP-12, no significant difference in the intensity of the bands between the major and minor alleles was observed (data not shown). No shifted band was observed in the case of SNP-1 and SNP-11. In the case of SNP-9, the band corresponding to the minor allele was more intense than that corresponding to the major allele, as observed in SNP-2 (Fig. 2B). The combination of the results of the luciferase assay and the gel-shift assay suggested that the genetic variations corresponding to SNP-2 and -9 were the most likely candidates to affect the transcriptional activity of *SCG3* and perhaps susceptibility to the development of obesity.

Expression of SCG3 in the hypothalamus

SCG3 was reported to be expressed in the hypothalamus, but its physiological roles have not yet been clarified (12). To further elucidate this role, we performed *in situ* hybridization and immuohistochemical analysis for *SCG3* in the murine hypothalamus and observed that *SCG3* was expressed in the LHA, PVN, ventromedial hypothalamus, and ARC (data not shown). SCG3 immunoreactivity was also observed in various other regions of the mouse brain as reported previously (12); however, the most intense immunoreactivities were observed in the ARC and LHA as well as the PVN and ventromedial hypothalamus (Fig. 3). The ARC neurons that express and secrete NPY and POMC are regulated by leptin and transfer their neuronal signal to orexin-expressing neurons in the LHA (26). To investigate the relationship between SCG3 and these neuronal peptides, we performed double-labeling immunohistochemical analysis and found that SCG3 was coexpressed with POMC and NPY in ARC cells (Fig. 3). We also examined the relationship between SCG3 and two major neuropeptides in the LHA that inhibit food intake, orexin and MCH, and detected that many orexin-expressing neurons and MCH-expressing neurons coexpressed SCG3 (Fig. 3).

Granins, such as CHGA, CHGB, and secretogranin II, form granule-like structure when they are expressed in cultured cells (11, 27). To examine whether SCG3 would also form granule-like structures and interact with each of these neuropeptides, we transfected pBI-*SCG3*-preproorexin, pBI-*SCG3*-pro-*MCH*, pBI-*SCG3*-*POMC*, and pBI-*SCG3*-pro-*NPY* into established BE(2)-C cell lines that were stably transfected with the pTet-Off vector system. The results indicated that SCG3 formed granule-like structures, like other granins, and colocalized with orexin, MCH, NPY, and POMC (Fig. 4). Immunoelectron microscopic analysis revealed that the granules were detected in BE(2)-C cells transfected with *SCG3* but not in those transfected with vector alone (data not shown). The granules stained with anti-SCG3 antibody (data not shown), suggesting that SCG3 forms secretory granules in neuroblastoma cells. These *in vivo* and *in vitro* data suggest that SCG3 may play some role in the secretion of neuropeptides that are related to appetite.

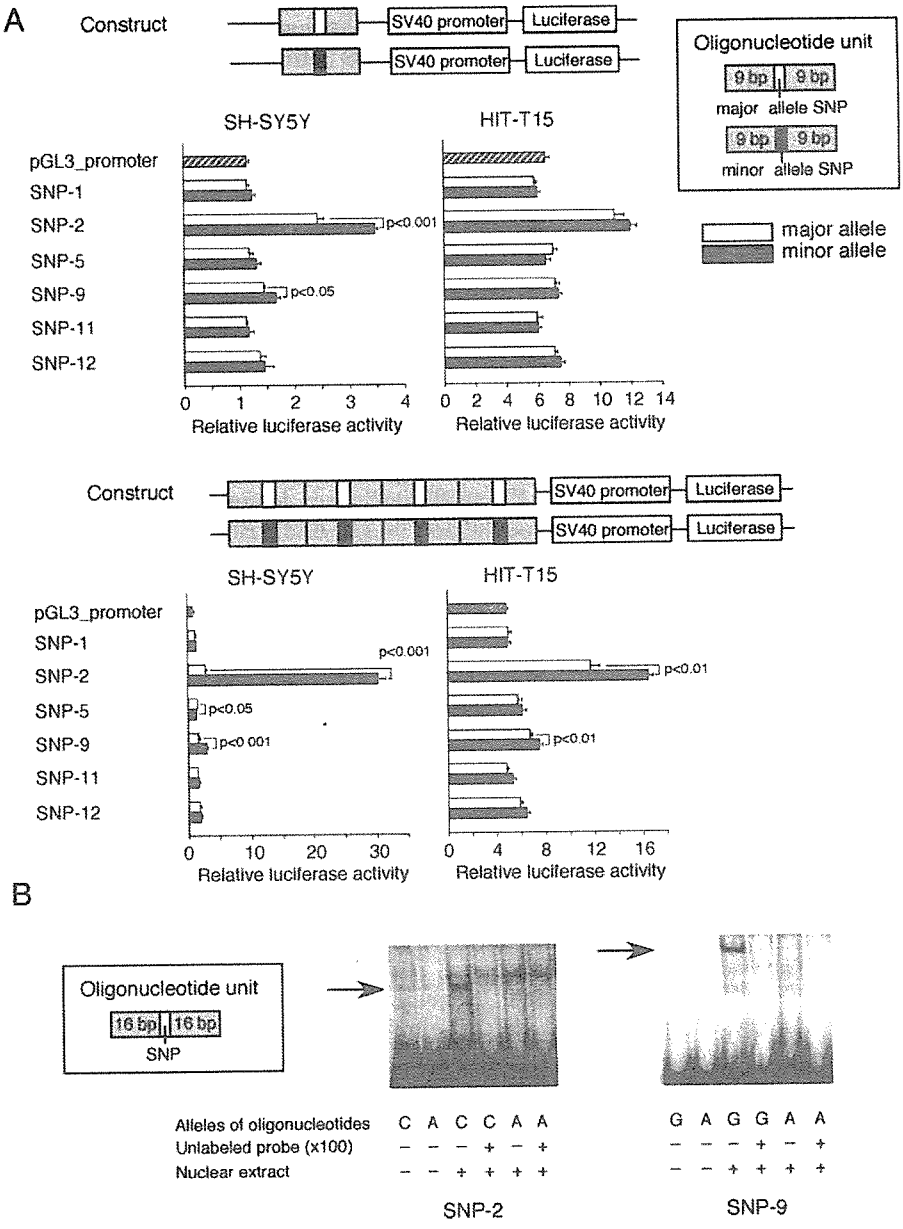

FIG. 2. Transcriptional activities affected by SNPs. A, Comparison of allelic variants of *SCG3* analyzed by relative luciferase activity in SH-SY5Y cells and HIT-T15 cells. The values are mean ± SD. pGL3-promoter, the empty vector. The *gray boxes* indicate the oligonucleotide unit around the SNPs, and *white* and *black small boxes* represent major and minor allele of each SNP, respectively. SV40, Simian virus 40. B, Binding of unknown nuclear factor(s) to the *SCG3* gene. Gel-shift assay was performed with digoxigenin-labeled 33-bp oligonucleotides corresponding to two *SCG3* polymorphic sites (SNP-2 and SNP-9) in SH-SY5Y cells. An *arrow* indicates the band that shows binding of nuclear proteins to the oligonucleotides containing minor alleles of SNP-2 (C allele, *left panel*) and SNP-9 (G allele, *right panel*).

Analysis of various quantitative phenotypes with SNP-2 and SNP-9

Because *SCG3* is expressed in pancreatic β-cells and involved in insulin secretion (13), SCG3 may play a role in metabolic disorders as well as in obesity. Therefore, to investigate whether the genotypes of SNP-2 and SNP-9 are related to the phenotypes of the metabolic disorders, we compared BMI, blood insulin, glucose, cholesterol, triglycerides, and high-density lipoprotein-cholesterol, and blood pressure among the different genotypes in cases and controls. We detected no relationship between these quantitative phenotypes and the genotypes at SNP-2 and SNP-9 in either the case or control groups.

The most important phenotype of the metabolic syndrome is visceral fat accumulation. Thus, we performed multiple linear regression analysis to further define the role of this gene in the amount of visceral and/or sc fat. The SNP-2 genotype was transformed to a multidichotomous variable, *i.e.* homozygosity with the A alleles *vs.* the other genotypes, heterozygosity *vs.* the other genotypes, or homozygosity with the C alleles *vs.* the other genotypes. Stepwise multiple regression analysis (both forward selection and backward elimination) revealed that gender, age, and BMI were significantly associated with VFA. However, no genotypes were significantly associated with VFA. In contrast, gender, BMI, and genotype (homozygosity with the A allele or heterozygosity with A and C alleles) were significantly associated with SFA. Neither age nor homozygosity with the C allele was significantly associated with SFA. Table 2 shows the data of multiple regression analysis using gender, age, BMI, and genotype as independent variables. Among the independent variables, homozygosity with the A allele, female gender, and increase in BMI were significantly associated with increases in SFA. In concordance, each of the three parameters,

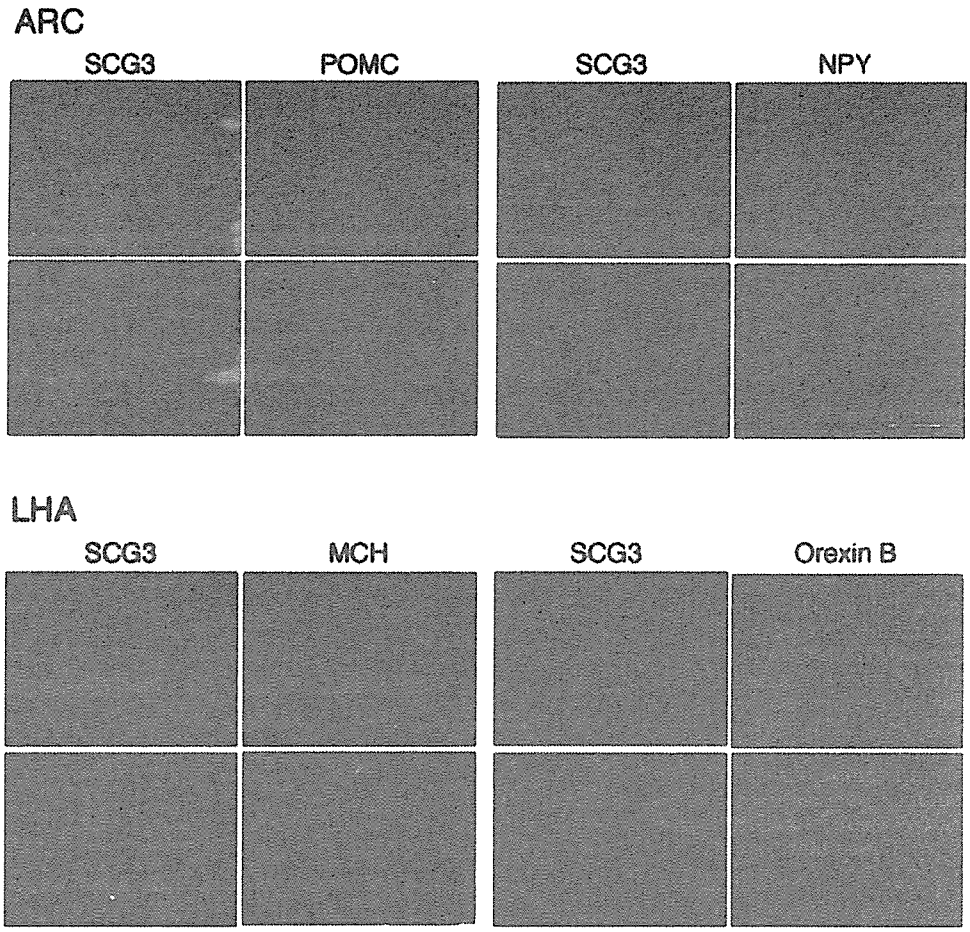

FIG. 3. Colocalization of SCG3 with POMC, NPY, MCH, or orexin in mouse hypothalamus. Immunostained tissue sections were double labeled for SCG3 with POMC, NPY, MCH, or orexin B. *Scale bar*, 50 μm.

heterozygosity with A and C alleles, male gender, and decrease in BMI, was significantly associated with a decrease in SFA. Because the number of homozygotes with the C allele was very small (n = 9), we were unable to validate its association with either SFA or VFA. SNP-2 and SNP-9 were in complete linkage disequilibrium (Δ = 1.0); thus, the same results were observed. These data suggested that the genotypes of SNP-2 and SNP-9 have an effect on the amount of SFA independent of the effects of the other independent variables.

Discussion

Epidemiological studies have provided evidence indicating the involvement of genetic factors in the development of obesity (5, 6). Through case-control association studies using gene-based SNPs, our center has successfully discovered candidate genes that confer susceptibility to various common diseases (myocardial infarction, diabetic nephropathy, type 2 diabetes mellitus) (9, 28–30). Using this approach, we identified novel functional SNPs associated with obesity, which are located in the *SCG3* gene. Our approach should prove effective and useful in searching for genes related to common diseases; however, the set of SNPs that we used covered only 11,932 gene loci. Recently a haplotype map of the human genome has been constructed (31). Despite the relatively high SNP density in genomic region, our SNP set only covered approximately 30% of the human genome by counting Hap-Map phase II SNPs that are: in LD ($r^2 > 0.5$) at least with one SNP in our set, with minor allele frequencies greater than 0.05, and at distances less than 500 kb from at least one SNP in our set. Because of this low genomic coverage for studies up to now, further investigations will be necessary as high-throughput genotyping products achieve higher SNP densities.

Intracellular granins are costored and cosecreted with peptide hormones (11). Our results suggest that SCG3 forms secretory granules together with orexin, MCH, NPY, and POMC in the hypothalamus. We demonstrated that SNP-2 and SNP-9 might have an effect on the transcriptional activity of the *SCG3* gene. Transcriptional activity of the major allele, the frequencies of which were higher in obese subjects than normal controls, was shown to be lower, which indicates that decreased *SCG3* expression levels may increase the risk of obesity. These results seem to be complicated. Many granins are known to work as inhibitors of endocrine secretion (11); for example, extracellular CHGA undergoes proteolytic processing into several bioactive peptides such as pancreastatin and catestatin (11). Pancreastatin inhibits insulin secretion from pancreatic β-cells (32), and catestatin inhibits the release of catecholamines from sympathoadrenal chromaffin cells (33). CHGA also inhibits POMC-derived peptide secretion (34). CHGB-derived peptides inhibit the secretion of PTH and insulin (35, 36). A secretogranin II-derived peptide, secretoneurin, inhibits serotonin and melatonin release from

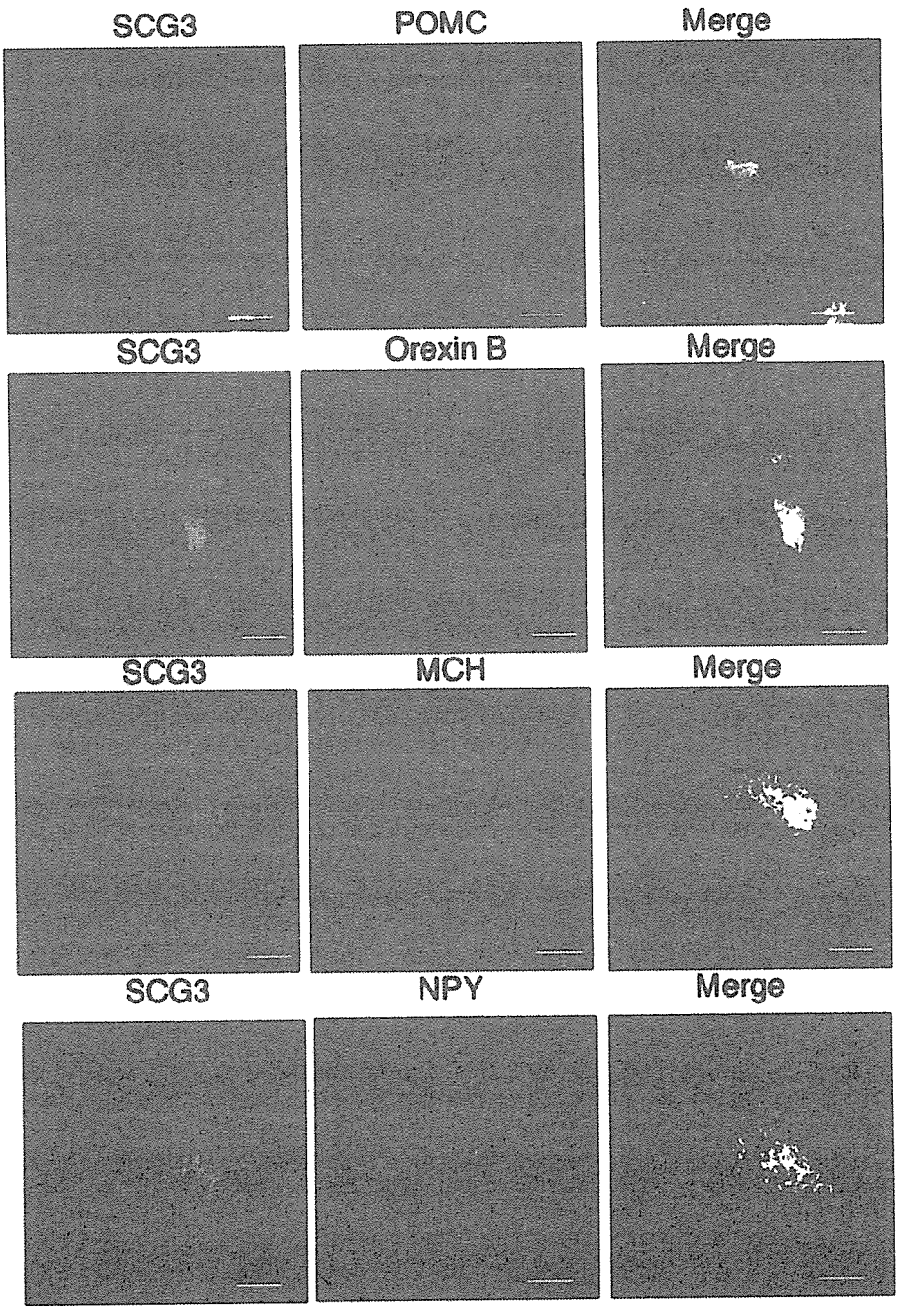

FIG. 4. Granular accumulation of SGG3 protein overexpressed in BE(2)-C cells. BE(2)-C cells expressed SCG3 (*green stain*) and POMC (*red stain*), NPY (*red stain*), orexin (*red stain*), or MCH (*red stain*). *Scale bars*, 10 μm.

pinealocytes (37). SCG3 also undergoes proteolytic processing and is secreted from cells (38). It needs to be investigated whether the peptides derived from proteolytic processing of SCG3 are bioactive and whether they may also inhibit the secretion of orexin, MCH and NPY, like other granins. Hence, we consider that increased expression of SCG3 in the subjects with the minor allele of SNP-2 and SNP-9 may result in a decrease in the secretion of orexin, MCH, and NPY and thereby inhibit food intake and accumulation of sc fat.

Food intake control is complicated (26) because in addition to many neuropeptides in the central nervous system, peptides secreted from other tissues, such as adipose tissue and gastrointestinal organs, participate in the control of food intake. The neural circuits in the hypothalamic region are also complicated, and the whole network is not well understood. There have been no reports indicating the involvement of SCG3 in appetite regulation, but in light of our data, it is interesting to speculate that SCG3 may be a potential factor in the regulation of food intake. Nevertheless, because fat accumulation is also affected by other variables like physical activity as well as food intake, it is also necessary to investigate whether SCG3 interacts with other variables.

Functional SNP-2 and SNP-9, which we have shown to be associated with obesity, are located on the chromosome 15q21 locus in which a positive linkage to SFA was indicated using the Québec Family Study (39). In concordance with this previous result, our study showed an association of SNP-2 and SNP-9 with SFA.

TABLE 2. Multiple linear regression analysis for VFA or SFA using SNP-2 (5′ flanking −1203) and other features as independent variables

Independent variables	AA *vs.* the other genotype			AC *vs.* the other genotype			CC *vs.* the other genotype		
	Regression coefficient	SE	*P*	Regression coefficient	SE	*P*	Regression coefficient	SE	*P*
VFA (dependent variable)									
Gender (men/women, 1/0)	61.271	6.583	<0.0001	61.398	6.572	<0.0001	61.352	6.570	<0.0001
Age (yr)	1.064	0.264	<0.0001	1.063	0.264	<0.0001	1.06	0.264	<0.0001
BMI (kg/m^2)	5.723	0.454	<0.0001	5.728	0.454	<0.0001	5.705	0.456	<0.0001
Genotype (1/0)	3.193	7.332	0.66	−2.293	7.577	0.76	−8.755	21.229	0.68
R^2	41%			41%			41%		
SFA (dependent variable)									
Gender (men/women, 1/0)	−69.550	7.167	<0.0001	−68.998	7.157	<0.0001	−67.623	7.244	<0.0001
Age (yr)	−0.329	0.289	0.26	−0.326	0.289	0.26	−0.357	0.293	0.22
BMI (kg/m^2)	13.044	0.495	<0.0001	13.095	0.496	<0.0001	12.998	0.503	<0.0001
Genotype (1/0)	25.499	7.952	0.0015	−25.761	8.221	0.0019	−11.438	23.275	0.62
R^2	67%			67%			66%		

In summary, we identified the genetic variations in *SCG3* that may influence the risk of obesity (particularly sc fat obesity) by a large-scale case-control association study. We found that SNP-2 and SNP-9 posses moderate effect sizes (supplemental Table 1) and affect the expression levels of SCG3 and that SCG3 forms secretory granules with hypothalamic neuropeptides. Our present data suggest that *SCG3* is a good target for the development of new medicine to aid in the prevention and treatment of obesity.

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Address all correspondence and requests for reprints to: Kikuko Hotta, Laboratory for Obesity, SNP Research Center, RIKEN, 1-7-22, Suehiro, Tsurumi-ku, Yokohama, Kanagawa 230-0045, Japan. E-mail: kikuko@src.riken.jp.

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REGULAR ARTICLE

Impact of weight reduction on production of platelet-derived microparticles and fibrinolytic parameters in obesity ☆

Takashi Murakami [a], Hitoshi Horigome [a,*], Kiyoji Tanaka [b], Yoshio Nakata [b], Kazunori Ohkawara [c], Yasutomi Katayama [c], Akira Matsui [a]

[a] *Institute of Clinical Medicine, University of Tsukuba, 1-1-1 Tennodai, Tsukuba, Ibaraki 305-8575, Japan*
[b] *Institute of Health and Sport Sciences and Center for Tsukuba Advanced Research Alliance (TARA), University of Tsukuba, Tsukuba, Ibaraki, 305-8574, Japan*
[c] *Graduate School of Comprehensive Human Sciences, University of Tsukuba, Tsukuba, Ibaraki 305-8574, Japan*

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Obese;
Weight reduction

Abstract

Introduction: Generation of platelet-derived microparticle (PMP) is implicated in cardiovascular disease (CVD). However, the influence of adiposity and weight reduction on PMP generation remains to be fully elucidated. We compared PMP generation and fibrinolytic parameters between 49 non-diabetic obese (obese group) and 37 age-matched non-obese subjects (control group), and compared the effects of weight reduction on the parameters between a 12-week calorie restricted diet and diet with aerobic exercise in obese subjects.
Materials and methods: PMP, plasma levels of plasminogen activator inhibitor-1 (PAI-1) activity and tissue-type plasminogen activator (t-PA) antigen were measured before and after intervention.
Results: Before intervention, PMP, PAI-1 activity and t-PA antigen values were elevated in the obese group compared with the control group. In all 86 subjects of both groups, these three parameters correlated with body mass index, waist circumference and fat tissue mass. There was a positive correlation between plasma levels of fibrinolytic parameters and visceral fat area (VFA). PMP values correlated with subcutaneous fat area (SFA). The intervention significantly reduced PMP, PAI-1

☆ This work was conducted at the Institute of Health and Sport Sciences, University of Tsukuba, and all the listed co-authors approve the content of the manuscript and submission to the Journal.

* Corresponding author. Tel.: +81 298 53 3210; fax: +81 298 53 3214.
E-mail address: hhorigom@md.tsukuba.ac.jp (H. Horigome).

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activity and t-PA antigen levels. There was a significant correlation between percentages of changes in PMP values and those in BMI, fat tissue mass and VFA in the obese group. No additional effect of exercise on PMP or fibrinolytic parameters was observed.
Conclusions: Overproduction of PMP and fibrinolytic abnormalities may be associated with excessive adipose tissue. Weight reduction by either calorie restriction with or without exercise improves fibrinolytic abnormalities and PMP overproduction, probably through reduction of adipose tissue.

Introduction

The prevalence of obesity continues to increase at an alarming rate worldwide [1]. Obesity is often associated with serious medical problems, such as impaired glucose tolerance, hyperinsulinemia, diabetes mellitus, hypertension, dyslipidemia, respiratory problems, premature atherosclerosis, and imparts a high risk of mortality from cardiovascular disease (CVD) [2,3]. Several studies have demonstrated that increased plasma levels of plasminogen activator inhibitor-1 (PAI-1) and tissue-type plasminogen activator (t-PA) are independent predictors of CVD [4–7], and that these parameters are implicated in the development of atherothrombosis in this population. Weight reduction contributes to a decrease in CVD-related morbidity through improvement of fibrinolytic abnormality and endothelial dysfunction [8–11]. Dietary therapy, physical activity and combination therapy (diet and physical activity) have been adopted as weight reduction regimens [2]. However, no studies have been conducted to compare the effects of simple calorie restriction and those of calorie restriction combined with aerobic exercise on fibrinolytic and endothelial parameters.

Recently, platelet derived microparticles (PMP) have been implicated in the development and/or progression of atherosclerosis [12]. PMP is a membrane vesicle released from activated platelets [13], and is considered to play an important role in the normal haemostatic response to vascular injury, since these particles exhibit prothrombinase and proinflammatory activities [14–20]. Increased levels of PMP have been observed in diabetes mellitus [21,22]. However, the association between PMP generation and adiposity is not fully understood. To the best of our knowledge, there are no reports that have assessed the effects of weight reduction on PMP levels in non-diabetic obese individuals. In the present study, we evaluated the relationship between PMP production and plasma fibrinolytic markers with adiposity levels in obese non-diabetic subjects. We also assessed the effect of calorie restricted diets and calorie restricted diets combined with aerobic exercise on PMP production and fibrinolytic markers.

Materials and methods

Subjects

We studied obese (body mass index (BMI) $\geq$25 kg/m^2) adult volunteers recruited from two local communities, Toride and Akeno, Ibaraki prefecture, Japan, via poster and local newspaper advertisements. Subjects with a history of taking antiplatelet drugs or a history of thromboembolic disease or intracranial hemorrhage or diabetes mellitus were excluded from the study. The 60 obese subjects were randomly assigned to one of the two groups (diet group and diet plus aerobic exercise group) of 12-week intervention. Eleven subjects withdrew from the study for unspecified reasons. Consequently, 49 non-diabetic obese participants (diet group, 9 females, 12 males; diet plus exercise group, 15 females, 13 males) aged 30–66 years (median, 52 years) completed this study. In 49 obese subjects (obese group), anthropometric measurement and blood samples were obtained at the time of entry into the weight reduction study and at completion of the 12-week intervention study. In 37 age-matched, non-obese (BMI; 19.1–24.9 kg/m^2) healthy subjects without a history of taking antiplatelet drugs or a history of thromboembolic disease, intracranial hemorrhage or diabetes mellitus (control group), aged 30–65 years (median, 53 years), these measurements and blood sampling were performed only once. We compared anthropometric and hematological parameters between non-diabetic obese group and control group at baseline, and compared the effect of weight reduction on the parameters between a calorie restricted diet group and diet with aerobic exercise group.

The study protocol was approved by the Human Investigation Review Committee of the University

of Tsukuba. All subjects gave their informed, written consent.

Anthropometric measurement, blood pressure and body composition analysis

Anthropometric parameters including body weight, height and waist circumference were measured in each case. BMI was calculated as weight in kilograms divided by height in square meters. Blood pressure was measured using a mercury manometer while the participants were sitting for at least 10 min in a chair. Body composition measurement for determination of fat tissue mass, was performed using bioelectrical impedance method (Omron, HBF-300, Kyoto, Japan).

Quantification of abdominal adipose tissue by CT

The visceral fat area (VFA) (cm^2) and subcutaneous fat area (SFA) (cm^2) were determined with a computerized tomography (CT) (Somaton AR.C, Siemens, Erlangen, Germany). The images were acquired at the level of L4–L5 in the supine position. The VFA and SFA were calculated using a computer software program (FatScan, N2system, Osaka, Japan) as described previously [23].

Blood collection and analysis

Blood samples were collected from the antecubital vein between 8 and 11 am after an overnight fast and at rest using the two-syringe method. The first sample was drawn into polypropylene tube for serum collection. The second sample was gently introduced into two polypropylene tubes containing 1/10 volume of 3.13% sodium citrate; one for platelet-poor plasma (PPP), obtained by centrifugation at 2000 $\times g$ for 10 min at 4 °C and stored at −80 °C until analysis, and one for platelet-rich plasma (PRP), obtained by centrifugation at 200 $\times g$ for 10 min at room temperature.

Total cholesterol, triglycerides, and high-density lipoprotein-cholesterol (HDL) and, low-density lipoprotein-cholesterol (LDL) were measured from serum samples using routine laboratory techniques. The levels of PAI-1 activity and tissue-type plasminogen activator (t-PA) antigen were determined from the PPP samples using a commercial kit (Hyphen BioMed for PAI-1 and t-PA, France). Fibrinogen plasma levels were quantified using standard laboratory method.

With PRP samples, PMP was analyzed using FACScan flow cytometer with CellQuest software (Becton Dickinson, San Jose, CA) using fluorescein isothiocyanate (FITC)-conjugated monoclonal antibody (mAb) to CD41 (Dakocytomation, Denmark). PRP samples (100 μl) were fixed with an equal volume of 2% paraformaldehyde and incubated at room temperature. Fixed PRP samples (20 μl) were then incubated with 4 μg/ml of mAb in the dark for 30 min at room temperature. Microparticles were identified by gating on CD41-positive events and distinguished from normal-sized platelets based on forward scatter size analysis (10,000 positive platelet events were analyzed). PMPs were reported as a percentage of the total platelet events.

Diet and exercise program

All subjects were instructed to take a balanced energy-restricted diet. The mean daily caloric intake was approximately 1200 kcal/day, ranging from 1000 to 1500 kcal/day for women, and 1680 kcal/day, ranging from 1500 to 1700 kcal/day for men. The subjects maintained a detailed record of food intake, and also received weekly nutritional counseling. Subjects in the diet plus exercise group performed aerobic exercise 3 days per week (60 min per session), supervised by physical trainers. The target exercise intensity was set at the level that raises heart rate to the predicted anaerobic threshold, or 12 (strong) to 14 (very strong) Borg scale.

Statistical analysis

Normally distributed data are presented as mean ± SEM and variables with skewed distribution are presented as median (p25–p75). Percentages of changes in variables were calculated as differences in values between after and before intervention, divided by the values before intervention. Statistical analysis was carried out using the unpaired *t*-test (for normally distributed data), Mann–Whitney test or Welch's test (for data that did not show normal distribution) for comparisons of baseline measurements between the groups, and the percentages of changes within the 12-week interval between the two intervention groups. Interval differences within the non-diabetic obese group were tested using the paired *t*-test (for normally distributed data) or Wilcoxon's signed-rank test (for data that did not show normal distribution). Relationships between the two measurements were assessed using Pearson's correlation coefficient test or Spearman's rank correlation where appropriate. A p value<0.05 (by two-tailed testing) was

Table 1 Baseline clinical characteristics

	Control group	Obese non-diabetic group	
n (F:M)	37 (21:16)	49 (24:25)	*p*
Age, year	49.2 ± 1.8	50.6 ± 1.4	.534
BMI, kg/cm^2	22.8 ± 0.2	27.4 ± 0.3	<.001
Waist circumference, cm	87.9 ± 0.8	96.6 ± 0.8	<.001
Fat tissue mass, kg	15.7 ± 0.6	22.1 ± 0.5	<.001
Visceral fat area, cm^2	78.0 ± 8.7	122.8 (77.3–162.9)	<.001
Subcutaneous fat area, cm^2	137.6 ± 10.8	217.6 ± 10.2	<.001
Systolic blood pressure, mm Hg	122.3 ± 1.8	131.9 ± 2.6	.003
Diastolic blood pressure, mm Hg	78.7 ± 1.4	85.0 ± 1.6	.004
Fibrinogen, mg/dl	242.7 ± 8.0	302.2 ± 8.9	<.001
Total cholesterol, mg/dl	214.4 ± 5.6	215.9 ± 5.4	.851
Triglycerides, mg/dl	78.0 (57.0–136.0)	106.0 (67.0–143.0)	.098
HDL-cholesterol, mg/dl	61.0 (52.0–75.0)	55.3 ± 1.7	.026
LDL-cholesterol, mg/dl	131.5 ± 6.1	136.1 ± 4.8	.551
Fasting glucose, mg/dl	88.9 ± 1.4	96.3 ± 1.5	.001
Hemoglobin A1c, %	4.89 ± 0.1	5.00 (4.80–5.60)	.033
PAI-1 activity, ng/ml	0.42 (0.18–0.57)	0.54 (0.34–1.05)	<.001
t-PA antigen, ng/ml	5.47 ± 0.3	6.73 (5.34–7.78)	.009
PMP, %	2.89 (2.16–4.73)	5.70 (4.07–7.22)	<.001

Data are mean ± SEM or median (p25–p75).

HDL, high-density lipoprotein; LDL, low-density lipoprotein; PAI-1, plasminogen activator inhibitor; t-PA, tissue-type plasminogen activator; PMP, platelet derived microparticle.

considered significant. All analyses were performed using StatView software (Macintosh).

Results

Baseline data

The baseline data of variables, including metabolic and anthropometric parameters of both groups are shown in Table 1. Compared with the control group, the subjects in the non-diabetic obese group had significantly higher plasma levels of PMP, PAI-1 activity and t-PA antigen.

The correlations between variables in all subjects of both control and obese groups at baseline are shown in Table 2. PAI-1 activity and t-PA antigen values correlated positively with BMI (Fig. 1), waist circumference, fat tissue mass and VFA. These fibrinolytic parameters also correlated positively with the plasma levels of triglyceride and hemoglobin A1c. An inverse correlation was noted between PAI-1 activity values and HDL-cholesterol values. PMP values correlated positively with BMI (Fig. 1), waist circumference, fat tissue mass and SFA. PMP levels did not correlate significantly with the levels of total cholesterol,

Table 2 Relationships among variables in all participants at baseline

	PAI-1 activity		t-PA antigen		PMP	
	r	*p*	*r*	*p*	*r*	*p*
Age, year	−.195	.080	.098	.383	−.083	.454
BMI, kg/cm^2	.353	.001	.341	.002	.536	<.001
Waist circumference, cm	.247	.030	.312	.006	.418	<.001
Fat tissue mass, kg	.337	.003	.253	.027	.506	<.001
Visceral fat area, cm^2	.348	.004	.370	.002	.240	.050
Subcutaneous fat area, cm^2	.059	.635	.226	.068	.390	.001
Systolic blood pressure, mm Hg	.051	.662	.070	.548	−.016	.887
Diastolic blood pressure, mm Hg	.055	.635	.114	.325	.037	.745
Fibrinogen, mg/dl	.222	.048	.238	.033	.134	.244
Total cholesterol, mg/dl	−.054	.633	.206	.067	.032	.773
Triglycerides, mg/dl	.279	.012	.243	.030	.120	.287
HDL-cholesterol, mg/dl	−.267	.017	−.169	.134	−.077	.496
LDL-cholesterol, mg/dl	−.063	.581	.230	.040	.002	.985
Fasting glucose, mg/dl	.191	.090	.248	.027	.196	.079
Hemoglobin A1c, %	.329	.003	.273	.016	.157	.166

HDL, high-density lipoprotein; LDL, low-density lipoprotein; PAI-1, plasminogen activator inhibitor; t-PA, tissue-type plasminogen activator; PMP, platelet derived microparticle.

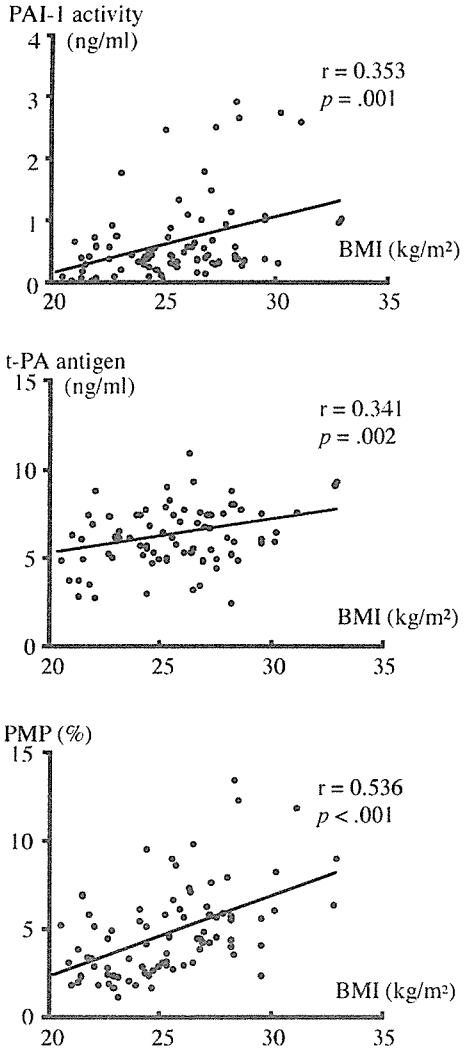

Figure 1 Relationships between BMI and plasma levels of PAI-1 activity, t-PA antigen and PMP. BMI, body mass index; PAI-1, plasminogen activator inhibitor-1; t-PA, tissue-type plasminogen activator inhibitor; PMP, platelet derived microparticle.

triglycerides, HDL-cholesterol, LDL-cholesterol, fasting glucose or hemoglobin A1c.

Before intervention (at baseline), there were no differences in distributions of age, sex, anthropometric parameters, blood pressure and body composition parameters between the diet group and diet plus exercise group (Table 3).

Effects of weight reduction in non-diabetic obese groups

After intervention, the 49 non-diabetic obese participants lost body weight with a mean loss of 8.04 ± 0.47 kg ($11.18 \pm 0.60\%$). The levels of PAI-1 activity, t-PA antigen and PMP were significantly reduced after the study (Table 3).

The relationships among percentages of changes in variables before and after 12-week intervention in 49 obese subjects are shown in Table 4. There was a positive correlation between percentages of changes in PAI-1 activity, t-PA antigen levels and those in BMI, waist circumference and fat tissue mass. A positive correlation was also noted between percentages of changes in PMP values and those in BMI, waist circumference, fat tissue mass and VFA (Fig. 2).

Comparison between diet group and diet plus exercise group

Percentages of change in BMI in the diet plus exercise group were significantly greater than those in the diet group after completion of the study. However, the type of intervention (diet vs. diet plus exercise) had no significant effect on the percentages of change in other parameters, including PAI-1 activity, t-PA antigen or PMP (Table 3).

Discussion

PMP: baseline data and effects of weight reduction

A definitive relationship between adiposity and PMP production in obesity is yet to be demonstrated. To our knowledge, this is the first report that investigates the association between adiposity and PMP levels and the effect of weight reduction on PMP production in non-diabetic obese individuals. In this study, PMP levels were significantly elevated in obese non-diabetic subjects in comparison to non-obese subjects, and the values correlated with BMI, waist circumference, fat tissue mass and SFA. Plasma levels of PMP correlated with VFA, although this correlation was not statistically significant ($r = 0.24$, $p = 0.050$). Significant reductions in PMP levels were observed after weight loss. Percentages of changes in PMP also correlated with those in BMI, waist circumference, fat tissue mass and VFA.

PMP is a membrane vesicle released upon platelet activation by agonists such as thrombin and collagen or by shear stress [13,14,24]. It has been reported that the high concentrations of prothrombin fragment 1+2 in obesity decrease after weight reduction [25]. Thrombin formation in obesity may play a role in PMP production, as in type 2 diabetes mellitus [22]. Recent studies revealed that adipose tissue produces bioactive molecules called "adipokines," such as tumor necrosis factor (TNF) and interleukin (IL)-6, that

Effects of 12-week program on various variables measured in the present study

	Diet group		Before and after 12 weeks	Diet plus exercise group		Before and after 12 weeks	Comparison b the two grou
	Baseline	Percentages of changes		Baseline	Percentages of changes		Variables at baseline
	21 (9:12)			28 (15:13)			
	51.0 (43.5–54.5)		*p*	52.5 (40.3–60.8)		*p*	*p*
γ^2	27.7 ± 0.5	−9.68 ± 1.0	<.001	27.3 ± 0.3	−12.12 ± 0.7	<.001	.444
ımference, cm	96.0 ± 1.5	−6.15 ± 3.7	<.001	97.1 ± 0.9	−8.13 ± 3.2	<.001	.505
mass, kg	21.7 ± 0.9	−20.75 ± 2.5	<.001	22.5 ± 0.5	−23.57 ± 1.6	<.001	.481
t area, cm^2	131.1 ± 14.2	−23.36 ± 3.3	<.001	125.9 ± 10.6	−29.76 ± 3.3	<.001	.718
ɔus fat area, cm^2	200.3 (150.9–244.5)	−22.31 ± 3.1	<.001	223.4 ± 12.4	−27.16 ± 2.1	<.001	.275
ɔod pressure, mm Hg	130.3 ± 3.2	−5.72 ± 1.9	.004	133.1 ± 3.8	−9.55 ± 1.8	<.001	.590
lood pressure, mm Hg	85.0 ± 2.4	−7.92 ± 1.7	<.001	85.0 ± 2.2	−11.22 (−19.2–6.7)	<.001	.997
, mg/dl	286.9 ± 9.3	−4.34 ± 2.6	.084	313.8 ± 13.7	−4.86 ± 3.7	.125	.110
ːsterol, mg/dl	210.5 ± 8.2	−6.75 ± 3.0	.011	219.9 ± 7.1	−10.50 ± 2.4	<.001	.392
es, mg/dl	107.0 (66.5–127.0)	−30.84 (−44.4–16.1)	.002	105.0 (64.5–176.8)	−30.90 ± 6.8	<.001	.770
ːterol, mg/dl	54.1 ± 2.3	6.53 ± 3.1	.037	56.2 ± 2.4	8.05 ± 2.6	.010	.554
terol, mg/dl	139.0 (113.0–157.0)	−5.45 ± 5.1	.040	138.2 ± 6.8	−9.89 ± 3.6	.005	.904
ıcose, mg/dl	93.4 ± 2.4	−2.10 ± 2.1	.246	98.4 ± 2.0	−4.83 ± 1.2	<.001	.113
n A1c, %	5.00 (4.75–5.65)	−1.54 ± 1.2	.284	4.95 (4.80–5.58)	−3.49 ± 1.3	.008	.951
ity, ng/ml	0.63 (0.33–1.05)	−37.84 (−80.6–4.55)	.015	0.47 (0.34–1.08)	−71.23 (−86.0–55.1)	<.001	.599
ːn, ng/ml	6.83 ± 0.4	−20.24 ± 8.6	.005	6.33 (5.25–7.78)	−29.21 ± 4.2	<.001	.572
	5.66 ± 0.6	−30.56 ± 4.9	<.001	6.13 ± 0.5	−35.67 ± 5.2	<.001	.538

ıean ± SEM or median (p25–p75).

density lipoprotein; LDL, low-density lipoprotein; PAI-1, plasminogen activator inhibitor; t-PA, tissue-type plasminogen activator; PMP, platelet derived micropa

Table 4 Relationships among percentages of changes in variables with 12-week interval

	PAI-1 activity		t-PA antigen		PMP	
	r	*p*	*r*	*p*	*r*	*p*
BMI	.495	<.001	.495	<.001	.341	.029
Waist circumference	.327	.023	.402	.004	.393	.010
Fat tissue mass	.451	.002	.392	.005	.418	.006
Visceral fat area	.270	.061	.185	.205	.485	.001
Subcutaneous fat area	.253	.080	.379	.007	.184	.250
Fibrinogen	−.110	.451	−.116	.435	−.222	.170
Total cholesterol	.117	.418	.058	.696	.303	.054
Triglycerides	.227	.116	.052	.723	.219	.170
HDL-cholesterol	−.116	.424	.165	.259	−.053	.744
LDL-cholesterol	.064	.655	.158	.281	.261	.100
Hemoglobin A1c	.381	.009	−.052	.718	−.010	.949

HDL, high-density lipoprotein; LDL, low-density lipoprotein; PAI-1, plasminogen activator inhibitor; t-PA, tissue-type plasminogen activator; PMP, platelet derived microparticle.

contribute to systemic and vascular inflammation [26]. The levels of these proinflammatory adipokines increase with increasing adipose tissue, and diminish after weight loss [27,28]. Nomura et al. [29] reported that IL-6 enhanced PMP production

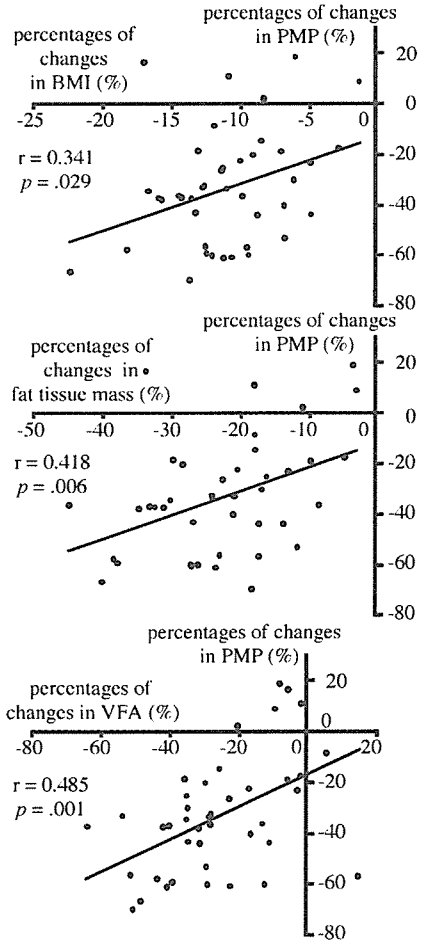

Figure 2 Relationships between percentages of changes in PMP levels and those in BMI, fat tissue mass and VFA within the 12-week period. PMP, platelet derived microparticle; BMI, body mass index; VFA, visceral fat area.

under high shear stress in patients with arteriosclerosis obliterans. In another study, PMP levels correlated with the levels of IL-6 and IL-8 in patients with systemic inflammatory response syndrome [30]. Furthermore, Diamant et al. [31] found that the values of various tissue factor-positive microparticle subpopulations correlate with BMI and TNF in uncomplicated type 2 diabetes mellitus, suggesting that low-grade inflammation, which is induced by adipose tissue, may enhance microparticle formation from various cell types. These facts support the idea that excessive adipocytes that lead to low-grade inflammation possibly play an important role in PMP production. Another possible explanation for PMP generation in obesity is the association of other adipokines such as leptin, because they increase in obesity and diminish after weight loss in proportion with BMI [25]. Additionally, high concentrations of leptin promote ADP-induced platelet aggregation, through leptin receptors expressed on the platelet surface [32,33]. Thus, increased level of leptin in obesity may influence ADP-induced PMP generation. Further studies are needed to determine the exact mechanism(s) of PMP generation in obesity.

The membrane of PMP is rich in negatively charged phospholipids, glycoprotein and P-selectin, which bind to coagulation factors [14–16], subendothelial matrix [17] and activated platelets and leukocytes [18]. PMP is thought to affect cell to cell interactions through the activation of monocytes and endothelial cells [19]. PMP also enhances the expression of adhesion molecules and cytokine production in THP-1 cells and endothelial cells [20]. Arachidonic acid is delivered to platelets and endothelial cells by PMP [34]. These procoagulant and inflammatory functions of PMP enhance the progression of atherosclerosis in non-diabetic obese individuals as well as in diabetic individuals [12]. Furthermore, tissue factor-positive microparticles

derived from platelets and T-helper cells are associated with components of the metabolic syndrome but not with coagulation markers. The above results suggest that tissue factor on microparticles may be involved in the processes of transcellular signaling or angiogenesis [31]. Thus, weight reduction may reduce the risk of CVD by decreasing PMP levels.

Fibrinolytic parameters: baseline data and effects of weight reduction

In this study, augmented plasma PAI-1 activity and t-PA antigen levels were observed in non-diabetic obese subjects. PAI-1 activity and t-PA antigen values positively correlated with BMI, fat tissue mass and VFA. These high values were reduced after weight reduction. A significant positive correlation between the percentages of changes in BMI, waist circumference and fat tissue mass and those in PAI-1 activity was observed. These associations were in line with data of previous weight reduction trials [8–10]. The fact that adipocytes are stimulated to produce PAI-1 by TNF, which is also secreted by adipocytes, further supports these associations [35].

Comparison between diet group and diet plus exercise group

Evidence from randomized trials shows that the initial goal of effective weight control therapies should be a reduction of body weight by approximately 10% from the baseline value [2]. From this point of view, the 12-week program used in the present study had a significant effect in non-diabetic obese individuals, since these subjects reduced their weight by $11.18 \pm 0.60\%$. Changes in the values of all parameters after weight reduction were favorable, with the exception of fibrinogen. Although additional effects of aerobic exercise on the percentages of changes in BMI were observed, changes in other parameters including fat tissue mass, VFA, PAI-1 activity, t-PA antigen or PMP values were comparable between the two intervention groups.

Regular physical activity seems to induce improvement in fibrinolytic activity, as indicated by an increase in t-PA activity and a decrease in PAI-1 activity [36]. Exercise at moderate intensity may also suppress platelet activation and polymorphonuclear leukocyte interaction with surface-adherent platelets under shear flow [37]. Despite these findings, several studies did not find any effect for the type of weight reduction program (dieting alone and dieting with aerobic exercise) on fibrinolytic parameters after weight reduction [8,9], as in our study. Further studies in a larger population are needed to endorse the additional effects of aerobic exercise on fibrinolytic capacity and platelet activity.

Conclusion

Our study indicates that excessive adipose tissue may induce overproduction of PMP and fibrinolytic abnormalities. No significant differences were observed in the effects of weight reduction on the associated hematological parameters between calorie-restriction diet with or without aerobic exercise groups. Weight reduction appears to be essential for the improvement of PMP overproduction and fibrinolytic abnormality, possibly through reduction of adipose tissue.

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原　著

中高齢者における血清高感度CRP濃度と運動耐容能の関連－SATプロジェクト188－

Relation between serum high-sensitive CRP concentration and exercise tolerance in middle-aged and elderly subjects －SAT project 188－

鰺坂隆一[*1]，大槻　毅[*2]，前田清司[*1,2]，松田光生[*2]
久野譜也[*1]，田中喜代次[*1]，曽根博仁[*1]

キー・ワード：risk factors for arteriosclerosis，exercise，C-reactive protein
動脈硬化危険因子，運動，C反応性蛋白

〔要旨〕新しい冠危険因子であるCRPと運動耐容能の関連は明らかではない．そこで，心疾患・呼吸器疾患を有さない中高齢者270名を対象に身体特性，生化学検査項目，症候限界漸増自転車エルゴメータ運動負荷試験による最高酸素摂取量を測定し，それらと血清高感度CRP濃度との関連を検討した．血清CRP濃度は0.637±0.715mg/Lであり，年齢，体重，BMI，インスリン，HDLコレステロール，最高酸素摂取量と有意の関連を認めた．ステップワイズ解析では最高酸素摂取量とBMIのみが有意に採択された．

以上から中高齢者において，運動耐容能はCRPの独立した関連因子である可能性が示唆された．

緒　言

C反応性たんぱく(CRP)は，体内の急性炎症に呼応してサイトカイン(とくにインターロイキン6：IL6)を介して非特異的に産生されるたんぱくであり，従来，急性炎症の指標として臨床的に用いられてきた[1]．最近，動脈硬化の進展における慢性血管炎症の意義が明らかになり，CRPの動脈硬化進展への関与を示唆する報告がなされている[2]．また，多くの疫学的研究により高感度法により測定された血清CRP濃度の上昇は他の危険因子とは独立した冠動脈疾患の危険因子であるとされている[3~5]．

一方，肥満に伴う脂肪組織におけるIL6の産生亢進によっても肝臓におけるCRP産生が促進されることから，血清CRP濃度はメタボリックシンドロームと密接な関連を有し，肥満の是正がその改善に有効であることが報告されている[6,7]．しかし，運動療法が減量効果と独立してCRPの改善に有効であるか否かについては一定の見解が得られていない[8~14]．また，中年成人において最大運動能と血清CRP濃度とが関連を有することが報告されているが，高齢者については報告がなく，亜最大運動能との関連も明らかではない[11,15~17]．さらに，肥満などの他のCRP関連因子と独立して運動耐容能が血清CRP濃度と関連を有するか否かについても一定の見解が得られていない[11,15~17]．

本研究の目的は，中高齢者において最大および亜最大運動耐容能が他の指標と独立して血清CRP濃度と関連を有するかを明らかにすることである．

*1　筑波大学大学院人間総合科学研究科
*2　筑波大学先端学際領域研究センター

表1　被験者のプロフィール

	男性	女性	全体
被験者数	103	167	270
年齢(歳)	69.9±5.4	65.9±6.8	67.4±6.6
身長(cm)	162.0±6.0	151.0±5.8	155.3±8.1
体重(kg)	61.9±8.1	52.9±6.5	56.3±8.4
BMI (kg/㎡)	23.5±2.6	23.2±2.8	23.3±2.7
肥満(人数)(%)	24(23.3%)	34(20.4%)	58(21.5%)
高血圧(人数)(%)	10(9.7%)	5(3.0%)	15(5.6%)
高脂血症(人数)(%)	27(26.2%)	68(40.7%)	95(35.2%)
糖尿病(人数)(%)	5(4.9%)	7(4.2%)	12(4.4%)
喫煙習慣(人数)(%)	21(20.4%)	3(1.8%)	24(8.9%)

BMI：体格指数(body mass index)

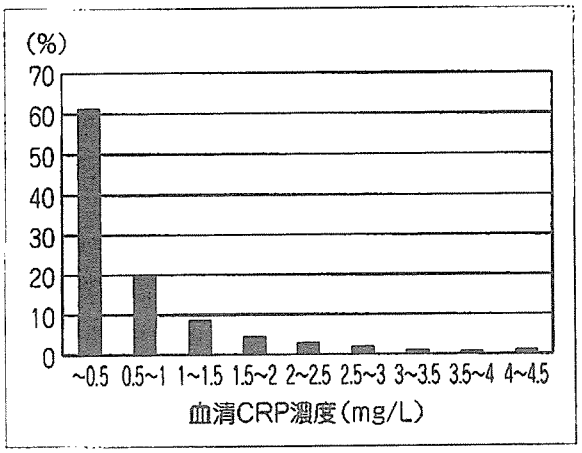

図1　血清CRP濃度分布
CRP血清濃度は0.05～4.29㎎/Lに分布し，平均0.637±0.715㎎/Lであった．220名(81.4%)が1㎎/L未満に分布していた．

方　法

1．対　象

対象は，地域広報誌などを用いてわれわれが主宰する運動教室(SATプロジェクト)へ参加した中高齢者のうち，心疾患・呼吸器疾患を有さず，服薬を全くしていない者である．血清CRP濃度は肝機能障害により上昇することが報告されている[18]ので，血清ALT濃度200IU/L以上の者も被験者に含めなかった．また，測定時に発熱などの症候があり，急性炎症の影響が否定できない被験者20名を検討から除外し，最終的な被験者数は270名であった．

本研究は筑波大学体育科学系研究倫理委員会の承認のもとに行い，各被験者には口頭および文書で説明し，同意を得た上で行った．

2．運動耐容能評価

運動耐容能は，同意の得られた238名において座位自転車エルゴメータを用い，最大運動耐容能または亜最大運動耐容能を評価した．最大運動耐容能は86名で評価した．被験者は座位にて15分以上の安静を保持した後に自転車エルゴメータ(エアロバイク800，Combi)に座り，さらに2分間の安静をとり，ウォーミングアップ運動(10W・2分間)に引き続き，ランプ負荷運動(10W/分)を行った．運動の中止基準は胸痛，呼吸困難感，心電図上の虚血性変化(J点より0.08秒の点での0.1mV以上のST下降もしくはJ点より0.04秒の点での0.1mV以上のST上昇)，危険な不整脈，高度の血圧上昇(収縮期血圧＞250㎜Hg)，自転車のペダル回転数を40/分以上に維持できない強い下肢疲労，年齢別予測最大心拍数(220－年齢)到達のいずれかとした．負荷試験中，呼気ガス分析装置(AE300S，ミナト医科学社製)を用いて酸素摂取量を1呼吸毎に測定し，最高酸素摂取量を最大運動耐容能の指標とした．152名の被験者においてはDPBP (double-product break point)法により亜最大運動耐容能を評価した．DPBPはTanakaほかの方法[19]に準じて決定し，DPBPを確実に超えたことを確認して自転車エルゴメータ運動を中止した．DPBP時酸素摂取量は以下の式を用いて算出し，体重あたりで評価した．

$$DPBP\text{-}\dot{V}O_2(ml/分)=10.594\times 仕事率(W)+357.97$$

表2　被験者の特性による血清CRP濃度の差異

	被験者数	血清CRP濃度(mg/L)	統計
男性	103	0.632±0.665	ns
女性	167	0.669±0.817	
中年者	88	0.490±0.693	p=0.0009
高齢者	182	0.735±0.782	
高血圧(+)	15	0.631±0.695	ns
高血圧(−)	255	0.656±0.766	
糖尿病(+)	11	0.771±0.456	p=0.0400
糖尿病(−)	259	0.650±0.772	
高脂血症(+)	95	0.725±0.888	ns
高脂血症(−)	175	0.617±0.683	
肥満(+)	58	0.903±0.949	p=0.0015
肥満(−)	212	0.587±0.689	
喫煙(+)	23	0.614±0.592	ns
喫煙(−)	247	0.659±0.776	
危険因子なし	73	0.350±0.304	p=0.0037
危険因子複合	5	1.002±0.538	

危険因子なし：中年者かつ肥満，糖尿病ともに保有なし．
危険因子複合：高齢者でかつ肥満，糖尿病ともに保有あり．

3．測定項目

a．罹病歴

問診表および健康診断データなどをもとにメディカルチェックを行い，高血圧，糖尿病，高脂血症，喫煙習慣などの血清CRP濃度に影響を及ぼす可能性のある項目の有無を調査した．これらの項目の有無の評価は循環器病の診断と治療のガイドライン合同研究班報告1999-2000年度版[20]に準拠した．本研究では総コレステロール220mg/dl以上，LDLコレステロール140mg/dl以上，HDLコレステロール40mg/dl未満，トリグリセリド150mg/dl以上のいずれかを満たす場合に高脂血症として一括して扱った．

b．身体特性

早朝空腹時に身長，体重を測定し，体格指数(BMI：body mass index)を算出した．

c．血液生化学検査

早朝安静空腹時に採血し，血清CRP濃度および糖・脂質代謝関連項目を測定した．CRPは高感度法であるラテックスネフロメトリー法にて測定した．

4．統計処理

数値は平均±標準偏差で示した．2変数間の関連の検討には線形回帰および非線形回帰(対数)による単相関分析を行ったが，結果がほぼ同じであったので，線形回帰を採用しPearsonの積率相関係数を求めた．さらに，血清CRP濃度を従属変数として，ステップワイズ回帰分析を行った．2群間の差の検定には，Wilcoxonの符号付順位検定，Mann-Whitney U検定またはFischer直接確率法を用いた．$p<0.05$をもって，有意とした．統計処理には，Statviewソフトウエア(SAS，USA)を用いた．

結　果

1．被験者のプロフィール

被験者のプロフィールを表1に示す．年齢は67.4±6.6(48～86)歳，性別は男性103名，女性167名であり，中年者(48～64歳)88名，高齢者(65～86歳)182名であった．表1に示したように肥満，高脂血症などの生活習慣病の保有者が多かった．

2．血清CRP濃度分布

血清CRP濃度は，0.05～4.29mg/Lに分布し，平均0.637±0.715mg/Lであった(図1)．220名(81.4%)が1mg/L未満に分布していた(図1)．

表3　血清CRP濃度と諸指標の関連

指標	相関係数(r)	有意差検定(p)
年齢	0.138	0.0238
身長	−0.101	ns
体重	0.183	0.0027
BMI	0.345	<0.0001
血糖	0.014	ns
HbA1c	0.047	ns
インスリン	0.187	0.0021
HOMA-R	0.171	0.0052
T-CHO	0.041	ns
HDL-CHO	−0.254	<0.0001
LDL-CHO	0.138	0.0234
TG	0.086	ns
peak $\dot{V}O_2$	−0.384	0.0003
DPBP $\dot{V}O_2$	−0.288	0.0003

BMI：体格指数(body mass index)，HOMA-R：HOMA指数，T-(HDL-，LDL-)CHO：総(HDL，LDL)コレステロール，TG：トリグリセリド，peak $\dot{V}O_2$：最高酸素摂取量，DPBP $\dot{V}O_2$：二重積(double product)変曲点(break point)における酸素摂取量

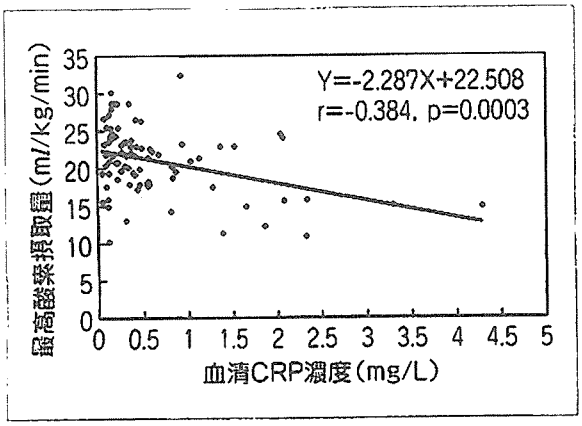

図2　血清CRP濃度と最高酸素摂取量の関連
両者間には有意の負の相関を認めた(r=−0.384，p=0.0003)．

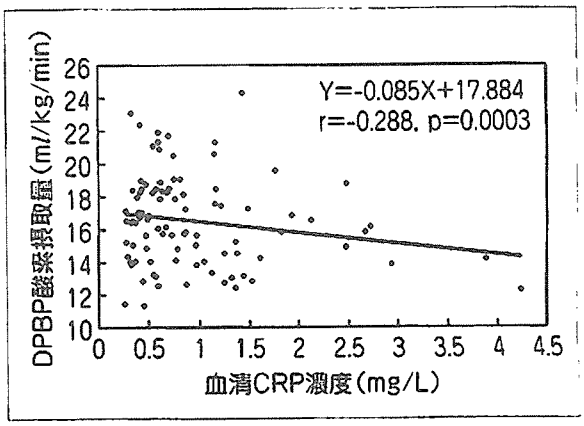

図3　血清CRP濃度とDPBP時点酸素摂取量の関連
両者間には有意の負の相関を認めた(r=−0.288，p=0.0003)．DPBP：二重積変曲点

3．血清CRP濃度と関連する因子

血清CRP濃度に男女差は認めなかった(表2)．一方，年代では中年者に比し高齢者において有意に高値であった(p=0.0009，表2)．

代表的な冠危険因子の有無による血清CRP濃度の差異を検討した．本研究では，糖尿病(p=0.0400)，肥満(p=0.0015)において有意差を認め，高血圧，高脂血症，喫煙習慣については差異を認めなかった(表2)．高脂血症については，総コレステロール，LDL-コレステロール，HDL-コレステロール，トリグリセリドについてそれぞれの異常の有無で個別に検討しても有意では無かった．血清CRP濃度を高める因子が複合した高齢かつ肥満・糖尿病保有者は中年かつ肥満，糖尿病を保有していない被験者に比べ3倍近い血清CRP濃度の上昇を認めた(p=0.0037，表2)．

血清CRP濃度と諸指標の単相関による関連の検討結果を表3に示す．従来の報告と同様に，年齢，体格指標，糖代謝，脂質代謝関連指標の多くと有意の関連を認めた(表3)．運動耐容能指標である最高酸素摂取量(r=−0.384)およびDPBP時点酸素摂取量(r=−0.288)とも有意の関連を認めた(図2，3)．中年者と高齢者それぞれにおいて検討しても中年者の方がより良好な関連であっ

たが，いずれの年代でも有意の関連を認めた(最高酸素摂取量：中年者 $r=-0.541$，$p<0.0001$，高齢者 $r=-0.297$，$p=0.0384$；DPBP時点酸素摂取量：中年者 $r=-0.447$，$p=0.0002$，高齢者 $r=-0.249$，$p=0.0105$)．

4．血清CRP濃度との関連－ステップワイズ回帰分析

単相関で有意の関連を認めた8指標を用いて，ステップワイズ解析を行った．最高酸素摂取量を測定した86名において，採用されたのは最高酸素摂取量とBMIのみであった($r=0.483$，$p<0.0001$)．一方，DPBP時点酸素摂取量で運動耐容能を評価した152名において採用されたのは，DPBP時点酸素摂取量，年齢，血清インスリン濃度，HOMA指数，血清HDLコレステロール濃度であり，BMIは採用されなかった($r=0.503$，$p<0.0001$)．

考　察

1．総　括

以上の成績を総合的に評価すると，中高齢者において，最大および亜最大運動耐容能が肥満などの他の因子とは独立した血清CRP濃度の関連指標であることが示唆された．

2．CRPの病態生理的・臨床的意義

C反応性たんぱく(CRP)は，体内の炎症に呼応して炎症性サイトカインを介して産生されるたんぱくである[1]．最近，動脈硬化部位における慢性血管炎症が血管内皮におけるCRPの産生を促進することが報告されている[2]．また，多くの疫学的研究により血清高感度CRP濃度の上昇は冠動脈疾患の危険因子であるとされている[3~5]．さらに最近，血清CRP濃度が冠動脈プラークの不安定性と関連する可能性も報告されている[21]．

したがって，運動療法により血清CRP濃度を低下させられれば冠動脈疾患の1次および2次予防に有益であると考えられる．一方，肥満に伴う脂肪組織におけるIL6の産生亢進によっても肝臓におけるCRP産生が促進されることから，肥満の是正がその改善に有効であることが報告されている[6,7]．しかし，運動療法が減量効果と独立してCRPの改善に有効であるか否かについては一定の見解が得られていない[8~14]．したがって，運動耐容能が肥満などの他のCRP関連因子と独立して血清CRP濃度と関連を有することが明らかとなれば，運動単独の効果を支持する傍証となりうると考えられる．

3．血清CRP濃度

多数の日本人中高齢者を被験者とした血清CRP濃度に関する先行研究によると，その平均値は男性では0.53，0.83，1.0，1.3 mg/Lであり，女性では，0.32，0.59，0.8，1.2 mg/Lである[22~25]．本研究の平均値は男性0.63mg/L，女性0.67 mg/Lであり，先行研究の成績の中間にある．これらの結果の差異はCRP測定法や測定条件(空腹時かそうでないか)の差異，急性炎症の除外を含む個々の被験者の特性の差異，服薬の有無，地域差などが考えられる．日本人においては，男性が女性より血清CRP濃度が高値を示す傾向が指摘されているが[24,25]，本研究では男女差を認めなかった．その原因の1つとして，血清CRP濃度を上昇させる喫煙習慣[26]を有する者が少なかったことが考えられる．

4．血清CRP濃度と関連する因子

日本人成人を対象とした研究において，血清CRP濃度と動脈硬化危険因子との関連が報告されている[23~26]．すなわち，年齢，BMI，体脂肪，収縮期および拡張期高血圧，血清HDLコレステロール濃度，血清トリグリセリド濃度は男女両方で，喫煙習慣は男性で，血清総コレステロール濃度と血糖は女性で，それぞれ血清CRP濃度と有意の関連があることが報告されている[23~26]．本研究においても，ほぼ同様の関連が認められた．先行研究との一部の不一致は前述のごとく，喫煙習慣を有する者が少ないこと，服薬者を除外したために高血圧罹患者が少ないことなどの被験者の特性の差異によると考えられる．

5．血清CRP濃度と運動の関係

身体活動量が少ない被験者において，血清CRP濃度が高いことが数多くの研究で示されている[15,16,25]．それらの研究では，身体活動量あるいはフィットネスはCRPだけでなく，白血球などの他の炎症マーカーとも有意の関連があることが示されており[25]，身体活動が何らかの機序で体内の慢性的炎症を軽減させて血清CRP濃度が低下することが示唆される．CRPの産生を促進するとされるサイトカインであるIL-6，IL-10の血中濃度も身体活動レベルの高い人で低値を示すことが報告されている[27]．

血清CRP濃度と運動耐容能との関連について