Table 7
BMD and other characteristics for men (n = 1108) according to *VLDLR* genotype

Characteristic	*SS*	*SL*	*LL*	*SS* + *SL*
Number (%)	154 (13.9)	517 (46.7)	437 (39.4)	671 (60.6)
Age (years)	59.4 ± 0.9	59.6 ± 0.5	58.6 ± 0.5	59.6 ± 0.4
Height (cm)	163.6 ± 0.5	164.6 ± 0.3	164.8 ± 0.3	164.4 ± 0.2
Body weight (kg)	61.1 ± 0.7	62.6 ± 0.4	62.7 ± 0.4	62.3 ± 0.4
BMD measured with pQCT (mg/cm^3)				
D50	261.8 ± 5.4	266.5 ± 2.9	269.2 ± 3.2	265.4 ± 2.6
D100	533.6 ± 7.5	539.7 ± 4.0	546.3 ± 4.4	538.3 ± 3.6
P100	1174.7 ± 11.3	1187.6 ± 6.1	1188.4 ± 6.6	1184.7 ± 5.4
BMD measured with DXA (g/cm^2)				
Total body	1.090 ± 0.007	1.083 ± 0.004	1.092 ± 0.004	1.085 ± 0.003
L2–L4	0.989 ± 0.012	0.968 ± 0.007[a]	1.000 ± 0.007	0.972 ± 0.006[a]
Femoral neck	0.745 ± 0.008	0.748 ± 0.004	0.760 ± 0.005	0.747 ± 0.004[b]
Trochanter	0.667 ± 0.008	0.663 ± 0.004	0.675 ± 0.005	0.664 ± 0.004[c]

BMD is adjusted for age, height, and body weight. Data are means ± SE.
[a] p = 0.002 versus *LL*.
[b] p = 0.032 versus *LL*.
[c] p = 0.048 versus *LL*.

In contrast to our observations for women, in a previous study [17] postmenopausal women with the *CC* genotype had a lower BMD at the femoral neck compared with those with the *TT* or the *TC* genotype. In a study of Danish women, among those with a body mass index of <25 kg/m^2, individuals with the *CC* genotype had a reduced BMD, bone mineral content, and cross-sectional area for the lumbar spine or femoral neck compared with those with the *T* allele, although these associations were not detected among women with a body mass index of ≥25 kg/m^2 [18]. In the present study, the *CC* genotype for the −34T → C polymorphism of *CYP17A1* was associated with increased BMD for postmenopausal women. The alleles of the −34T → C polymorphism associated with increased BMD thus differ between the present study (*C* allele) and previous studies (*T* allele) [17,18]. The reason for this apparent discrepancy is unclear. The distribution of −34T → C genotypes differed significantly between the postmenopausal Japanese women in our study (*TT*, 27%; *TC*, 49%; *CC*, 24%; n = 813) and the postmenopausal Caucasian women in the previous studies (*TT*, 42%; *TC*, 50%; *CC*, 8%; n = 252; $p < 0.0001$, χ^2 test [17]; and *TT*, 36%; *TC*, 49%; *CC*, 15%; n = 1788; $p < 0.0001$ [18]), possibly reflecting the difference in ethnicity. The difference in the effects of *CYP17A1* genotype on BMD between ethnic groups might be attributable either to a difference in lifestyle factors or gene–environment interactions or to linkage disequilibrium with other BMD susceptibility genes.

Association of the −493G → T polymorphism of MTP and the CGG repeat polymorphism of VLDLR with BMD

Bone loss is associated with an expansion of adipose tissue in bone marrow [19], and osteoblasts and adipocytes share a

Table 8
Effects of genotypes for *CYP17A1*, *MTP*, and *VLDLR* on BMD

Genotype	D50	D100	P100	Total body	L2–L4	Femoral neck	Trochanter
All women							
CYP17A1	0.761	0.827	0.291	0.223	0.041 (0.002)	**0.006** (0.004)	0.108
MTP	0.955	0.171	0.988	0.922	0.730	0.091	0.495
VLDLR	0.499	0.040 (0.002)	0.057	0.154	0.471	0.205	0.116
Premenopausal women							
CYP17A1	0.855	0.435	0.243	0.838	0.107	0.550	0.862
MTP	0.019 (0.019)	**0.003** (0.029)	0.039 (0.013)	0.172	0.843	0.045 (0.012)	0.157
VLDLR	0.708	0.553	0.411	0.906	0.552	0.500	0.333
Postmenopausal women							
CYP17A1	0.655	0.968	0.127	0.222	0.192	**0.005** (0.006)	0.085
MTP	0.334	0.759	0.580	0.846	0.115	0.207	0.618
VLDLR	0.421	0.098	0.174	0.118	0.794	0.242	0.178
Men							
CYP17A1	0.748	0.486	0.025 (0.004)	0.173	0.241	0.150	0.119
MTP	0.670	0.951	0.481	0.600	0.609	0.807	0.592
VLDLR	0.355	0.159	0.666	0.232	**0.003** (0.007)	0.034 (0.003)	0.044 (0.003)

Data were analyzed by multiple regression analysis including age, height, body weight, and genotype for *CYP17* (*TT* = *TC* = 0, *CC* = 1), *MTP* (*GG* = *GT* = 0, *TT* = 1), or *VLDLR* (*SS* = *SL* = 0, *LL* = 1). Data are p values (R^2). p values <0.01 are shown in bold.

Table 9
Effects of serum lipid profile on BMD

Serum lipid profile	D50	D100	P100	Total body	L2–L4	Femoral neck	Trochanter
Women							
Total cholesterol	**<0.001** (0.022)	**<0.001** (0.020)	**<0.001** (0.021)	**<0.001** (0.041)	**<0.001** (0.031)	**<0.001** (0.028)	**<0.001** (0.014)
HDL-cholesterol	0.124	**<0.001** (0.013)	**<0.001** (0.015)	**<0.001** (0.015)	0.383	0.251	0.309
LDL-cholesterol	**<0.001** (0.017)	**<0.001** (0.018)	**<0.001** (0.021)	**<0.001** (0.042)	**<0.001** (0.025)	**<0.001** (0.019)	**0.002** (0.008)
Triglycerides	**<0.001** (0.013)	**<0.001** (0.032)	**<0.001** (0.031)	**<0.001** (0.035)	**0.003** (0.008)	**<0.001** (0.012)	**<0.001** (0.009)
Men							
Total cholesterol	0.014 (0.005)	**0.006** (0.007)	0.090	0.704	0.350	0.981	0.507
HDL-cholesterol	0.775	0.855	0.893	0.204	0.028 (0.004)	**<0.001** (0.010)	**0.002** (0.008)
LDL-cholesterol	**0.007** (0.007)	0.012 (0.006)	0.041 (0.004)	0.397	0.838	0.117	0.046 (0.004)
Triglycerides	0.518	0.513	0.953	0.274	0.624	0.333	0.273

Data were analyzed by simple regression analysis. Data are p values (R^2). p values <0.01 are shown in bold.

common progenitor derived from stromal cells in bone marrow [20]. Products of lipoprotein oxidation and an atherogenic diet also inhibit preosteoblast differentiation and result in reduced bone mineralization [20,21]. In addition, lipid-lowering agents (statins) stimulate bone formation and inhibit bone resorption, resulting in the prevention of both bone loss and osteoporotic fractures [22]. Early postmenopausal women with an atherogenic serum lipid profile (total cholesterol of ≥240 mg/dl, LDL-cholesterol of ≥160 mg/dl, or lipoprotein(a) of ≥25 mg/dl) had a lower BMD for the lumbar spine or femoral neck and an increased risk of osteopenia compared with those with a normal lipid profile [23]. Postmenopausal women with increased plasma concentrations of LDL-cholesterol have also been shown to be at greater risk of developing osteopenia than are those with normal concentrations, suggesting that an increased plasma level of LDL-cholesterol is a risk factor for reduced BMD [24]. These observations suggest the existence of a close relation between lipid and bone metabolism as well as demonstrating adverse effects of an atherogenic lipid profile on bone remodeling.

Although hyperlipidemia has been shown to be related to reduced BMD or osteoporosis [23,24], the mechanism that underlies this relation remains unknown. A contributing factor might be the fact that estrogen deficiency results both in impairment of lipid metabolism and in acceleration of bone resorption [23]. Alternatively, bone formation might be impaired by ischemia of bone tissue caused by dyslipidemia, given that osteoblast progenitors are located adjacent to the subendothelial matrix of bone vessels and atherosclerosis may influence the function of these bone-forming cells [23]. Another possibility relates to the potential interaction between cholesterol synthesis and regulation of bone metabolism, given that statins have beneficial effects on bone mass [22].

MTP mediates the transport of triglycerides, cholesteryl esters, and phospholipids between phospholipid surfaces [26]. The −493G → T polymorphism of *MTP* has been associated with the plasma concentration of LDL-cholesterol [28], with the *T* allele being related to low concentrations. However, other studies have failed to detect an association between this polymorphism and the plasma concentration of LDL-cholesterol [41] or have detected an adverse effect of the *T* allele [42]. This discrepancy might be related to the observation that the phenotype associated with the −493G → T polymorphism of *MTP* is modulated by visceral obesity and hyperinsulinemia [43]. In the present study, BMD for the distal radius was significantly greater in premenopausal women with the *TT* genotype than in those with the *G* allele. *MTP* genotype was not, however, associated with the serum concentrations of total cholesterol, HDL-cholesterol, LDL-cholesterol, or triglycerides in women. It is thus unlikely that the association of this polymorphism with BMD in premenopausal women was attributable to an effect on serum lipid concentrations. The mechanism by which the −493G → T polymorphism of *MTP* affects BMD thus remains unclear.

VLDL-R is a member of the LDL-R family of proteins, is abundant in fatty acid-metabolizing tissues, binds triglyceride-rich lipoproteins (but not LDL), and functions as a peripheral receptor for remnant lipoproteins [44]. VLDL-R-deficient mice manifest a reduced adipose tissue mass compared with wild-type mice [45]. The CGG repeat polymorphism of *VLDLR* has been associated with the prevalence of sporadic Alzheimer disease in Japanese [34] and with that of vascular dementia [46]. It has also been shown to be related to the plasma concentrations of lipoproteins, with the $(CGG)_{n=9}$ allele being associated with lower levels of lipoprotein E:B and higher levels of lipoprotein A-I [33]. The frequencies of the $(CGG)_{n=9}$ and $(CGG)_{n=5}$ alleles were lower and higher, respectively, in subjects with dyslipidemia treated with lipid-lowering drugs than in control subjects, although the statistical significance of this difference was marginal (p = 0.054) [33]. In the present study, the combination of two $(CGG)_{n\geq 8}$ alleles was significantly associated with increased BMD for the lumbar spine in men. There were, however, no differences in the serum concentrations of total cholesterol, HDL-cholesterol, LDL-cholesterol, or triglycerides among *VLDLR* genotypes in men. It is thus unlikely that the association of the CGG repeat polymorphism with BMD in men was attributable to an effect on serum lipid concentrations. The effects of the CGG repeat polymorphism of *VLDLR* on gene expression or the function of the encoded protein have not been determined.

The molecular mechanism of the effect of this polymorphism on BMD thus remains unclear.

There are some limitations of the present study: (1) Serum concentrations of vitamin D were not measured in the study population. Although no subjects with clinical vitamin D deficiency, such as osteomalacia, were included, the National Nutrition Survey in 2001 suggested that vitamin D intake was smaller than the daily requirement (100 IU) in ~25% of Japanese individuals. The serum concentration of free thyroxine slightly exceeded the normal range (0.77 to 1.93 ng/dl) in three subjects. It is thus possible that individuals with subclinical vitamin D deficiency or thyrotoxicosis were included in the present study. (2) Given that the effects of single polymorphisms on BMD were small, the association between a polymorphism and BMD might be influenced by age, gender, or status of sex hormones. The associations observed in the present study were thus not apparent in the entire population, only in subgroups. (3) Given the multiple comparisons of genotypes with BMD, we adopted a strict criterion ($p < 0.01$) for statistical significance. However, we did not perform Bonferroni's correction. It is therefore not possible to rule out completely the occurrence of potential statistical errors such as false positives. (4) It is possible that the polymorphisms examined in our study are in linkage disequilibrium with polymorphisms of nearby genes that are actually responsible for regulating BMD.

Despite these various limitations, our present results suggest that *CYP17A1* and *MTP* are susceptibility loci for increased BMD in postmenopausal and premenopausal Japanese women, respectively, and that *VLDLR* constitutes such a locus for Japanese men.

Materials and methods

Study population

The National Institute for Longevity Sciences Longitudinal Study of Aging is a population-based prospective cohort study of aging and age-related diseases [47]. The subjects are stratified by both age and gender and are randomly selected from resident registrations in the city of Obu and town of Higashiura in central Japan [48,49]. The lifestyle of residents of this area is typical of that of Japanese individuals. The numbers of men and women recruited are similar and age at the baseline is 40 to 79 years, with similar numbers of participants in each decade (40s, 50s, 60s, 70s). The subjects will be followed up every 2 years. All participants are subjected at a special center to a detailed examination, which includes not only medical evaluation but also assessment of exercise physiology, body composition, nutrition, and psychology. Individuals who had disorders known to cause abnormalities of bone metabolism, including diabetes mellitus, chronic renal failure, rheumatoid arthritis, as well as parathyroid, thyroid, adrenal, and other endocrine diseases, or who had taken drugs that affect bone metabolism, such as estrogen, glucocorticoids, bisphosphonates, vitamin D, and statins, were excluded from the present study. Individuals whose genotypes were not successfully determined (two individuals for *CYP17A1*, 27 individuals for *VLDLR*) were also excluded from the analysis.

We examined the relations of BMD at various sites to the −34T → C polymorphism of *CYP17A1* (NCBI, dbSNP, rs743572) in 2230 individuals (1108 women, 1122 men), to the −493G → T polymorphism of *MTP* (NCBI, dbSNP, rs1800591) in 2232 individuals (1108 women, 1124 men), and to the triplet repeat [$(CGG)_n$] polymorphism of *VLDLR* (GenBank Accession No. D16495) in 2205 individuals (1097 women, 1108 men). In addition, to uncover potential differences between women according to menopausal status, we conducted all analyses separately for premenopausal and postmenopausal women. Menopausal status was evaluated with a detailed questionnaire, and menopause was defined as complete cessation of menstruation. The study protocol complies with the Declaration of Helsinki and was approved by the Committee on Ethics of Human Research of the National Institute for Longevity Sciences. Written informed consent was obtained from each subject.

Measurement of BMD

BMD at the radius was measured by peripheral quantitative computed tomography (pQCT) (Desiscan 1000; Scanco Medical, Bassersdorf, Switzerland) and was expressed as D50 (distal radius BMD for the inner 50% of the cross-sectional area, comprising mostly cancellous bone), D100 (distal radius BMD for the entire cross-sectional area, including both cancellous and cortical bone), and P100 (proximal radius BMD for the entire cross-sectional area, consisting mostly of cortical bone). BMD for the total body, lumbar spine (L2–L4), right femoral neck, and right trochanter was measured by dual-energy X-ray absorptiometry (DXA) (QDR 4500; Hologic, Bedford, MA, USA). The coefficients of variation of the pQCT instrument for BMD values were 0.7 (D50), 1.0 (D100), and 0.6% (P100), and those of the DXA instrument were 0.9 (total body), 0.9 (L2–L4), 1.3 (femoral neck), and 1.0% (trochanter).

Determination of genotype

Genotypes for *CYP17A1* and *MTP* were determined with a fluorescence-based allele-specific DNA primer assay system (Toyobo Gene Analysis, Tsuruga, Japan) [50]. The polymorphic region of *CYP17A1* was amplified by the polymerase chain reaction (PCR) with sense primers labeled at the 5′ end with either fluorescein isothiocyanate (5′-GCCACAGCTCTTCTACTCCAXCG-3′) or Texas red (5′-TGCCACAGCTCTTCTACTCCAXTG-3′) and with an antisense primer labeled at the 5′ end with biotin (5′-ATAAGCTAGGGTAAGCAGCAAGAGA-3′). The polymorphic region of *MTP* was amplified with allele-specific sense

primers labeled at the 5′ end with either fluorescein isothiocyanate (5′-ACATTATTTTGAAGTGATTGGX*T*G-3′) or Texas red (5′-ACATTATTTTGAAGTGATTGGX-*G*G-3′) and with an antisense primer labeled at the 5′ end with biotin (5′-AATTCACACTGAATTTTAGGATTTA-3′). The reaction mixture (25 μl) contained 20 ng of DNA, 5 pmol of each primer, 0.2 mmol/L of each deoxynucleoside triphosphate, 3 (for *CYP17A1*) or 4.5 (for *MTP*) mmol/L $MgCl_2$, and 1 U of rTaq DNA polymerase (Toyobo, Osaka, Japan) in polymerase buffer. The amplification protocol comprised initial denaturation at 95°C for 5 min; 35 cycles of denaturation at 95°C for 30 s, annealing at 65 (for *CYP17A1*) or 62.5°C (for *MTP*) for 30 s, and extension at 72 (for *CYP17A1*) or 70°C (for *MTP*) for 30 s; and a final extension at 72 (for *CYP17A1*) or 70°C (for *MTP*) for 2 min. The amplified DNA was then incubated in a solution containing streptavidin-conjugated magnetic beads in the wells of a 96-well plate at room temperature, and the plate was placed on a magnetic stand. The supernatants from each well were transferred to the wells of a 96-well plate containing 0.01 mol/L NaOH and were measured for fluorescence with a microplate reader (Fluoroscan Ascent; Dainippon Pharmaceutical, Osaka, Japan) at excitation and emission wavelengths of 485 and 538 nm, respectively, for fluorescein isothiocyanate and of 584 and 612 nm, respectively, for Texas red.

The triplet repeats [$(CGG)_n$] in the 5′ untranslated region of *VLDLR* were amplified by PCR with a sense primer (5′-CTCCCTTCCCCCGCCAACTC-3′) and with an antisense primer labeled at the 5′ end with 6-carboxyfluorescein (5′-GCCCAGAGCGCGGACGTG-3′). The reaction mixture (25 μl) contained 20 ng of DNA, 5 pmol of each primer, 0.2 mmol/L of each deoxynucleoside triphosphate, 1 mmol/L $MgSO_4$, and 0.4 U of KODplus DNA polymerase (Toyobo) in polymerase buffer. The amplification protocol comprised initial denaturation at 94°C for 5 min; 40 cycles of denaturation at 94°C for 30 s, annealing at 65°C for 30 s, and extension at 68°C for 30 s; and a final extension at 68°C for 2 min. The size of triplet-repeat-containing DNA fragments amplified by PCR was determined with a Prism 3100 DNA sequencer with GeneScan and Genotyper software (Applied Biosystems, Foster City, CA, USA).

Measurement of serum lipid profile

Venous blood was collected in the early morning after the subjects had fasted overnight. Blood samples were centrifuged at 1600 *g* for 15 min at 4°C, and serum was separated and stored at −30°C until analysis. The serum concentrations of total cholesterol, HDL-cholesterol, LDL-cholesterol, and triglycerides were measured by standard methods.

Statistical analysis

Statistical analysis was performed with SAS software (SAS Institute, Cary, NC, USA). Data were compared among three groups by one-way analysis of variance and the Tukey–Kramer post hoc test and between two groups by the unpaired Student *t* test. BMD values were compared among genotypes of each polymorphism with adjustment for age, height, and body weight by the least-squares method in a general linear model. Allele frequencies were estimated by the gene-counting method, and the χ^2 test was used to identify significant departure from Hardy–Weinberg equilibrium. The relation of the number of CGG repeats in *VLDLR* to BMD was analyzed by simple regression analysis. The relation of serum concentrations of total cholesterol, HDL-cholesterol, LDL-cholesterol, or triglycerides to BMD was also analyzed by simple regression analysis. The effect of genotype of each polymorphism on BMD was evaluated by multivariable regression analysis; p values and R^2 were calculated from analyses including age, height, body weight, and genotype for *CYP17A1* ($TT = TC = 0$, $CC = 1$), *MTP* ($GG = GT = 0$, $TT = 1$), or *VLDLR* ($SS = SL = 0$, $LL = 1$). A p value of <0.01 was considered statistically significant.

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研究報告●18

地域在住中高年者における転倒恐怖感の要因に関する縦断的検討

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1 背景と目的

転倒恐怖感とは，転倒するのではないかという不安感，恐怖感である。転倒恐怖感は，本来ならば遂行可能な日常生活を制限し，閉じこもりや寝たきりにつながる危険性もあることから，生活の質を低下させる重大な要因になると指摘されている[1,2)]。

最近の研究では，転倒経験以外にも，生活機能や抑うつなど，様々な身体的・心理的変数と転倒恐怖感との関連が示されている[3,4)]。しかしながら，これらのほとんどは横断調査の結果であり，転倒恐怖感と諸変数の因果関係は明らかにされていない。予防的観点の重要性を考えると，転倒恐怖感の先行要因について縦断的に検討する必要がある。

本研究では，地域在住中高年者における転倒恐怖感の推移，および転倒恐怖感の生起に関連する要因について，縦断的に検討する。

2 方法

1．対象

対象は，国立長寿医療センター研究所疫学研究部が行っている「老化に関する長期縦断疫学調査(National Institute for Longevity Sciences-Longitudinal Study of Aging (NILS-LSA))」の第1次調査(Wave 1：1997～2000年)，2年後の第2次調査(Wave 2：2000～2002年)にともに参加した50～79歳(Wave 1時)の地域在住中高年者1,299名(平均年齢62.9±8.1歳：男性695名，女性604名)である。なお，NILS-LSAは，年齢および性で層化無作為抽出された地域住民を対象とした老化と老年病に関する縦断的コホート調査であり，国立長寿医療センター倫理委員会の了承の下に，「調査への参加の文書による同意(informed consent)」の得られた者を対象として行われている[5)]。

2．変数

調査票により以下の変数を収集して，コーディングを行った。

1）Wave 1

転倒恐怖感(有(とても怖い・少し怖い)＝1，無(怖くない)＝0)，年代(50～64歳＝1，65～79歳＝0)，生活機能(老研式活動能力指標[6)]：低(≦10)＝1，高(11≦)＝0)，主観的健康感(不良(非常に悪い・悪い)＝1，良好(非常に良い・良い・普通)＝0)，抑うつ(老人用うつ尺度(GDS)[7)]：高(6≦)＝1，低(≦5)＝0)。

2）Wave 2

転倒恐怖感(有(とても怖い・少し怖い)＝1，無(怖くない)＝0)，過去1年間の転倒経験(有＝1，無＝0)，過去2年間の入院経験(有＝1，無＝0)，骨折経験(有＝1，無＝0)。

3．統計解析

転倒恐怖感無(Wave 1)の中高年者を対象として，転倒恐怖感(Wave 2)を結果変数，その他を説明変数として回帰分析を行った。具体的には，χ^2検定によって結果

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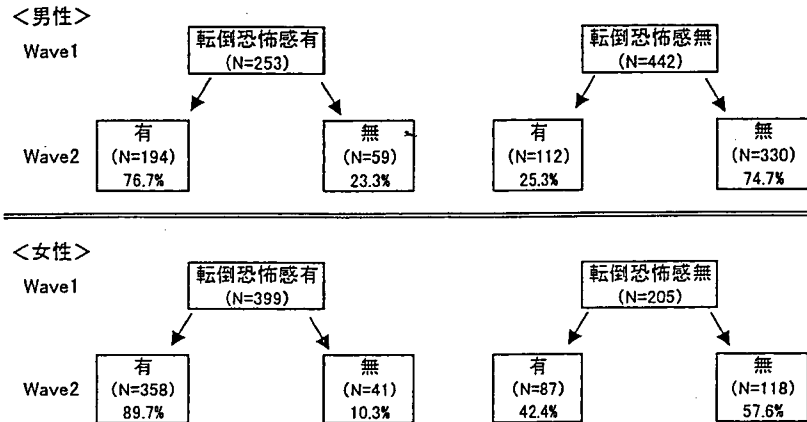

Fig.1 転倒恐怖感の推移

変数と各説明変数との関連性を検討し，有意な関連($p<0.10$)を示した変数を説明変数とするロジスティック回帰分析を行った。なお，これまでに転倒恐怖感の分布や関連要因に性差が確認されている[4]ことから，性別に解析した。統計解析にはSAS release 8.2を用いた。

3 結果

1．転倒恐怖感の推移(Fig.1)

転倒恐怖感無(Wave 1)のうち，転倒恐怖感有(Wave 2)に変化した中高年者は199名(30.8%)であった。性別にみると，男性では，転倒恐怖感無(Wave 1)442名中，転倒恐怖感有(Wave 2)は112名(25.3%)，女性では，転倒恐怖感無(Wave 1)205名中，転倒恐怖感有(Wave 2)は87名(42.4%)であった。

2．転倒恐怖感の生起に関連する要因(Table 1)

転倒恐怖感無(Wave 1)の中高年者を対象として，転倒恐怖感(Wave 2)を結果変数，その他を説明変数とするχ^2検定およびロジスティック回帰分析を性別に行った。

男性において，χ^2検定により転倒恐怖感(Wave 2)と有意な関連を示した変数は，年代・主観的健康感・転倒経験・入院経験であった。これらを説明変数としたロジスティック回帰分析(ステップワイズ法)を行った結果，年代(65～79歳)・主観的健康感(不良)(以上，$p<0.001$)・転倒経験(有)($p<0.01$)・入院経験(有)($p<0.05$)の場合に転倒恐怖感(Wave 2)を有する傾向が高かった。一方，女性において，χ^2検定により転倒恐怖感(Wave 2)と有意な関連を示した変数は，年代・転倒経験・骨折経験であり，ロジスティック回帰分析の結果，年代(65～79歳)($p<0.001$)・骨折経験(有)($p<0.05$)の場合に転倒恐怖感(Wave 2)を有する傾向が高かった。

Table 1 ロジスティック回帰分析結果
結果変数：Wave 2 転倒恐怖感(無＝0，有＝1)

	Odds ratio	95%CI
<男性>		
年代(65～79歳)	2.51***	1.56～3.96
主観的健康感(不良)	2.90***	1.32～6.37
転倒経験(有)	2.03**	1.16～3.56
入院経験(有)	2.09*	1.11～3.94
<女性>		
年代(65～79歳)	3.75***	1.99～7.05
骨折経験(有)	2.22*	1.04～4.74

***：p<0.001，**：p<0.01，*：p<0.05。
注）χ^2検定によって転倒恐怖感と有意な関連($p<0.10$)を示した項目を説明変数として分析を行った。

4 考察

2年の間に転倒恐怖感無から有へと移行した中高年者は，男性で25.3%，女性で42.4%であり，中高年期には特に女性で，転倒恐怖感を生起しやすいことが確認された。また，生起に関連する要因を検討した結果から，年代が高い場合に転倒恐怖感を生起する傾向が認められた。この結果は，転倒恐怖感と性別・年齢との関連を指摘する先行研究[1-4]の知見と一致している。さらに，ある時点において転倒恐怖感を有していなくても，男性では主観的健康感が不良であった場合や転倒，入院を経験した場合，女性では骨折経験があった場合に，その後，転倒恐怖感

有へと移行する可能性が高いことが明らかになった。転倒恐怖感を有する中高年者をスクリーニングしたり，転倒恐怖感の生起を抑制するための介入方法を検討する際には，これらの先行要因を考慮する必要があると考えられる。

5 結語

地域在住中高年者における転倒恐怖感について縦断的に検討した結果，2年の間に転倒恐怖感無から有へと移行する中高年者が存在すること，転倒恐怖感の生起に関連する男性・女性特有の要因があることが示された。

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PAPER

Effects of aerobic exercise and obesity phenotype on abdominal fat reduction in response to weight loss

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OBJECTIVE: To test the effects on abdominal fat reduction of adding aerobic exercise training to a diet program and obesity phenotype in response to weight loss.
DESIGN: A prospective clinical trial with a 14-week weight-loss intervention design.
SETTING AND PARTICIPANTS: In total, 209 overweight and obese women were assigned to four subgroups depending on type of treatment and the subject's obesity phenotype: diet alone (DA) with intra-abdominal fat (IF) obesity ($\geq$mean IF area), diet plus exercise (DE) with IF obesity, DA with abdominal subcutaneous fat (ASF) obesity ($<$mean IF area) and DE with ASF obesity. Abdominal fat areas were evaluated by CT scans, with values adjusted for selected variables.
RESULTS: Values were adjusted for age, menopausal status and change in body weight and total fat mass. The IF reductions were significantly ($P<0.0001$) greater in subjects with IF obesity phenotype ($-45.1\,cm^2$) compared to the ASF obesity phenotype ($-22.2\,cm^2$). The ASF reductions were significantly ($P<0.001$) greater for subjects with ASF obesity ($-74.5\,cm^2$) compared to IF obesity ($-55.5\,cm^2$). For IF obesity, the IF reduction was significantly ($P<0.01$) greater in the DE group ($-49.3\,cm^2$) than in the DA group ($-37.8\,cm^2$).
CONCLUSION: These results suggest that for individuals with IF obesity, the efficacy on reducing IF of adding aerobic exercise training to a diet-alone weight-reduction program is more prominent ($-49.3\,cm^2/-37.8\,cm^2=1.3$ times) compared with DA. Moreover, abdominal fat reduction was found to be modified by obesity phenotype in response to weight loss.
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Keywords: aerobic exercise; diet; weight loss; obesity phenotype; abdominal fat

Introduction

Obesity is closely associated with some major health risk factors,[1,2] and the prevalence of obesity continues to increase in developed countries.[3] It is well known that individuals with central (android-type) obesity are at greater risk for coronary heart disease (CHD) and several metabolic disorders.[4] Although waist circumference and waist-to-hip ratio continue to be widely used anthropometric indices for determining central fat obesity,[5,6] intra-abdominal fat (IF) measured by computerized tomography (CT) scans is also an index for evaluating abdominal adiposity. The IF accumulation is strongly associated with metabolic disorders independent of whole-body adiposity, including high blood pressure and triglycerides as well as an increased incidence of diabetes mellitus.[7–9]

Since it is known that weight-loss treatment benefits the health of obese individuals,[10–12] obese patients with risk factors for CHD should be treated by an appropriate weight-loss program. Unfortunately, losing weight through diet alone (DA) includes a decline in fat-free mass during the intervention period[12] and induces an attenuation of fat oxidation after the intervention period,[13] which may contribute to weight regain. To prevent regaining weight after weight loss, therefore, it has been recommended that obesity be treated with exercise training in addition to reduced energy intake.[14]

It is unclear whether aerobic exercise training improves total and abdominal obesity.[15,16] Several studies reported that aerobic exercise training, for example, walking, stair climbing, stationary cycling and aerobics, which were prescribed for treating obesity, do not always play an important role in weight loss.[17–19] Some studies have demonstrated that physical activity was associated with IF

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reduction,[20–23] but others found no association.[24,25] In addition, sample sizes of these studies were too small to provide conclusive evidence that exercise training actually reduces IF. A report from the National Institutes of Health states that there is insufficient evidence to determine whether an increase in physical activity is associated with a corresponding reduction in abdominal obesity in a dose–response manner.[26] Nevertheless, Shimomura *et al*[27] have found in rats that aerobic exercise training markedly reduced acyl-CoA synthetase activity and mRNA levels of acyl-CoA synthetase, lipoprotein lipase and GLUT-4 in mesenteric (intra-abdominal) fat. Acyl-CoA synthetase is a key enzyme for lipid accumulation in adipose tissue. Lipoprotein lipase and GLUT-4 are known to be two main metabolic steps for uptake of energy (lipid and glucose, respectively) to adipose tissue. Hence, the study by Shimomura *et al*[27] may provide evidence that there is a greater reduction in IF compared with subcutaneous fat after exercise training.

Our first aim in this study was to elucidate whether adding aerobic exercise training to a dietary weight-reduction program further reduces total and abdominal fat over DA in a large sample size of overweight and obese Japanese women. A review by Ross and Janssen[16] found that in studies reporting only minor reduction in IF in response to exercise training, baseline IF values were lower compared to studies reporting substantial reductions in IF. Therefore, we also tested an assumption that obesity phenotype (large IF *vs* small IF) may modify the reductions in IF and abdominal subcutaneous fat (ASF) in response to weight loss.

Subjects and methods

Subjects

In total, 303 women were recruited through advertisements in local newspapers. Through medical history and physical examination, we excluded individuals who were nonobese or nonoverweight (body mass index less than 25 kg/m^2),[28] smoked, had concomitant renal, hepatic or cardiac disease, or were being treated with drugs such as beta-blockers, which could affect the variables of the study. In total, 225 women, aged 21–66 y, were chosen as subjects. To increase subjects' adherence to the weight-reduction programs, the subjects' personal lifestyle (occupations, daily schedules, etc) was taken into account and they were placed in either a DA group ($n=73$) or a diet plus exercise (DE) group ($n=152$). Five subjects in the DA group and 11 in the DE group were unable to complete the study successfully for personal reasons. Consequently, 68 subjects in the DA group, consisting of 17 postmenopausal and 51 premenopausal women, and 141 subjects in the DE group, consisting of 46 postmenopausal and 95 premenopausal women were included in the final analysis. The subjects were divided by mean of IF area into two obesity phenotypes: IF obesity (IF area $\geq 104.7\,cm^2$, $n=105$) and ASF obesity (IF area $< 104.7\,cm^2$, $n=104$). Assays and measurements were carried out before and after the 14-week intervention period. The aim and design of the study were explained to each subject before they gave their written informed consent. This study was approved by the Higashi Toride Hospital Review Board.

Anthropometric variables

Body weight was measured to the nearest 0.1 kg using a digital scale, height was measured to the nearest 0.1 cm using a wall-mounted stadiometer, and BMI was calculated as weight (kg) divided by height squared (m^2).

Body composition by bioelectrical impedance analysis

Body composition, recorded as percentage fat mass, absolute total fat mass (kg) and fat-free mass (kg), was assessed by a bioelectrical impedance analysis at 50 kHz (SS-103, Sekisui, Tokyo, Japan).[29] Measurements were conducted with subjects in the supine position after at least a 20-min rest.

Abdominal adipose tissue area by CT

The IF and ASF areas (cm^2) were measured at the level of the umbilicus (L4-L5) using CT scans (SCT-6800TX, Shimadzu, Tokyo, Japan) performed on subjects in the supine position. The IF and ASF areas were calculated using a computer software program (FatScan, N2system, Osaka, Japan).[30] The intraclass correlation for repeated IF and ASF areas determinations in our laboratory are 0.99.

Assessment of CHD risk factors

Systolic and diastolic blood pressures were taken from the left arm using a sphygmomanometer after the subjects rested at least 20 min in a sitting position. Cuff sizes were selected based on upper arm girth and length. A blood sample of approximately 10 ml was drawn from each subject after an overnight fast. Serum total cholesterol and triglycerides were determined enzymatically, and fasting plasma glucose was assayed by a glucose oxidase method. Serum high-density lipoprotein-cholesterol was measured by the heparin-manganese precipitation method. Five criteria for CHD risk factors were defined: (1) systolic blood pressure $\geq$140 mmHg; (2) diastolic blood pressure $\geq$90 mmHg; (3) total cholesterol $\geq$5.70 mmol/l (220 mg/dl); (4) triglycerides $\geq$1.70 mmol/l (150 mg/dl); (5) fasting plasma glucose $\geq$7.00 mmol/l (126 mg/dl). Frequencies and percentages of subjects with high systolic blood pressure, diastolic blood pressure, total cholesterol, triglycerides and fasting plasma glucose were 75 (36%), 60 (29%), 95 (45%), 36 (17%) and 11 (5%), respectively.

Maximal oxygen uptake. Maximal oxygen uptake (VO_{2max}, ml/kg/min) was determined during a graded exercise test using a cycle ergometer (818E, Monark, Stockholm, Sweden). Following a 2-min warm-up, the subject started with a

workload of 15 W, which was increased by 15 W each minute until volitional exhaustion occurred. Pulmonary ventilation and gas exchange were measured breath-by-breath with an on-line data acquisition system (Oxycon alpha System, Mijnhardt, Breda, Netherlands).

Diet and exercise regimens

Dietary protocol. All subjects were instructed to take a well-balanced supplemental food product (*MicroDiet*, Sunny-Health, Nagano, Japan) every day. It was developed for very low-energy diets (170 kcal per pack) and is comprised of protein, carbohydrates, fat, various amino acids, vitamins and minerals. Two other meals per day were allowed consisting an average of 240 kcal of protein, 480 kcal of carbohydrate and 240 kcal of fat. Subjects also kept daily food diaries during the 14-week intervention period and learned about proper daily nutrition through weekly lectures and counseling by skilled dieticians. They were asked to record the brand name and amounts (in grams) of every food and beverage ingested with each meal. To calculate energy intake (in kilocalories) and the amounts of each nutrient (fat, protein, and carbohydrate in grams), data from 12 days (3 days/week: 2 weekdays and either Saturday or Sunday before the intervention period; 9 days: 3 days/months for 3 months during the intervention period) were randomly selected from the food diaries. Skilled dieticians analyzed the data (Table 1).

Exercise protocol. In addition to restricting energy intake, the subjects from the DE group performed a bench stepping exercise 3 days/week for 45 min per session supervised in the hospital by two or three physical trainers. The bench stepping exercise is a combination exercise of low impact aerobic dance and stepping with a step bench (10–20 cm high).[31] The exercise started with basic steps for the first four weeks and then progressed to combination of basic steps and lunge steps for the next six weeks, and finally progressed to more advanced lunge steps for the last four weeks. Subjects were instructed to perform the aerobic dance at a level that raised their heart rate to 70–85% of the corresponding heart rate at their VO_{2max}. The target Borg's scale (ratings of perceived exertion)[32] ranged from 13 (fairly hard) to 17 (very hard). The oxygen costs of the bench stepping exercise were measured three times (at first, middle and last month) during the study by the aforementioned metabolic measurement system. Moreover, they were instructed to walk every day around their houses for more than 30 min per session. The target exercise intensity was set at a level that raised their heart rate to 40–50% of the corresponding heart rate at their VO_{2max}, or 9 (very light) to 11 (light) of the Borg's scale. They were instructed how to measure their heart rates by palpation while walking every week. At that time, portable heart rate monitors monitored their heart rates. Hence, they could check the validity of their heart rates by palpation. They were asked to every day record the duration (min) and intensity (heart rate and the Borg's scale) of the walking each session. According to subjects' diary (body weight, heart rate and walking time), energy expenditure while walking was calculated every day each subject using below equation.[33]

Energy expenditure (kcal) = 5.04 (kcal) × VO_{2max} (ml/kg/min) × percentage heart rate reserve during walking × body weight (kg) × duration (min)/1000 ml.

Table 1 Dietary intake per day of subjects

	Diet alone (n = 51)		*Diet plus exercise (n = 70)*	
	Pretreatment	*Change*	*Pretreatment*	*Change*
	Mean ± s.d.	*Mean ± s.d.*	*Mean ± s.d.*	*Mean ± s.d.*
Energy, 8 (kcal)	1971 ± 405	−861 ± 359	1965 ± 343	−874 ± 400
Protein (g)	94 ± 136	−27 ± 135	90 ± 55	−23 ± 85
Fat (g)	59 ± 18	−22 ± 28	61 ± 16	−30 ± 19
Carbohydrate (g)	266 ± 67	−122 ± 69	274 ± 72	−133 ± 73

Statistical analysis

Values are expressed as mean ± standard deviations in tables and as mean ± standard errors in figures. Unpaired *t*-tests were used to test difference in changes in variables between the two treatment groups. The relationship between two measurement variables was assessed by Pearson's product moment correlation. Correlation coefficients were compared using a test based on *z*-transformed correlation coefficients. Multiple regression analyses with the forward stepwise method were performed to estimate the independent contribution of age, treatment, pre- or postmenopausal status, total fat mass change and baseline IF or ASF area to the variations in changes in IF and ASF area. General linear model analyses (repeated measure ANOVA with *post hoc* tests) were used to test for differences in dependent variables among four subgroups (DA and DE groups with IF obesity, and DA and DE groups with ASF obesity) with selected variables as covariates.

Analyses were performed on log-transformed values for the variables that were not normally distributed. Probability values below 0.05 were regarded as significant. The data were analyzed with the Statistical Analysis System (SAS), version 8.2 (SAS Institute Inc, Cary, NC, USA).

Results

Attendance at the bench stepping exercise (40 sessions) averaged 92% (range 83–100%) for the subjects from the DE group. There were 55 subjects whose diaries were available for calculating energy expenditure. The average VO_2 during the bench stepping exercise was 19.5 ± 4.7 ml/kg/min (first month 17.3 ± 2.6 ml/kg/min, second month 19.8 ± 3.1 ml/kg/min and last month 21.3 ± 3.6 ml/kg/min), which corresponded to 81.5 ± 14.7% of VO_{2max} (first month 72.4 ± 9.8%,

Table 2 Anthropometric variables, body composition, abdominal fat areas and CHD risk factors at pre- and post-treatments, and comparisons of changes in variables between groups with diet alone and diet plus exercise

	Intra-abdominal fat obesity				*Abdominal subcutaneous fat obesity*			
	Diet alone (n = 34)		*Diet plus exercise (n = 71)*		*Diet alone (n = 34)*		*Diet plus exercise (n = 70)*	
	Pretreatment	*Change*	*Pretreatment*	*Change*	*Pretreatment*	*Change*	*Pretreatment*	*Change*
	Mean ± s.d.	*Mean ± s.d.*	*Mean ± s.d.*	*Mean ± s.d.*	*Mean ± s.d.*	*Mean ± s.d.*	*Mean ± s.d.*	*Mean ± s.d.*
Bodyweight (kg)	71.5 ± 8.8	−7.0 ± 2.4	71.7 ± 8.1	−9.6 ± 3.3***	67.5 ± 6.9	−7.9 ± 3.6	67.0 ± 6.9	−7.9 ± 2.7
Bodymass index (kg/m²)	29.4 ± 3.2	−2.8 ± 1.0	29.5 ± 2.9	−3.9 ± 1.3***	27.8 ± 2.0	−3.1 ± 1.5	27.2 ± 2.1	−3.2 ± 1.1
Percentage fat mass (%)	36.6 ± 3.4	−3.6 ± 2.3	37.7 ± 5.2	−5.9 ± 3.6***	33.4 ± 3.5	−4.4 ± 2.5	33.8 ± 3.6	4.9 ± 2.6
Total fat mass (kg)	26.1 ± 3.8	−4.9 ± 1.9	27.2 ± 6.0	−7.3 ± 3.2***	22.7 ± 4.0	−5.3 ± 2.5	22.7 ± 4.0	−5.5 ± 2.2
Fat-free mass (kg)	45.3 ± 6.2	−2.1 ± 1.8	44.4 ± 4.6	−2.2 ± 2.3	44.8 ± 4.1	−2.7 ± 2.1	44.3 ± 4.3	−2.5 ± 1.5
VO_{2max} (ml/kg/min)	25.0 ± 3.3	2.6 ± 2.8	24.5 ± 4.1	5.2 ± 4.2***	26.2 ± 4.2	3.0 ± 3.5	27.5 ± 4.6	5.1 ± 4.5***
IF area (cm²)	148 ± 41	−37 ± 19	135 ± 27	−52 ± 28**	68 ± 24	−23 ± 17	69 ± 21	−21 ± 15
ASF area (cm²)	266 ± 62	−44 ± 32	289 ± 90	−69 ± 40**	264 ± 49	−67 ± 43	246 ± 64	−70 ± 42
Systolic blood pressure (mmHg)	138 ± 15	−10 ± 14	140 ± 19	−13 ± 11	129 ± 18	−10 ± 11	129 ± 18	−11 ± 12
Diastolic blood pressure (mmHg)	86 ± 10	−4 ± 9	85 ± 11	−8 ± 9*	84 ± 9	−9 ± 8	81 ± 11	−8 ± 10
Total cholestrol (mmol/l)	5.9 ± 0.8	−0.5 ± 0.8	5.8 ± 0.8	−0.5 ± 0.7	5.4 ± 0.7	−0.3 ± 0.5	5.6 ± 1.0	−0.5 ± 0.8
Triglycerides (mmol/l)	1.4 ± 0.7	−0.4 ± 0.5	1.5 ± 0.6	−0.6 ± 0.5	1.1 ± 0.5	−0.2 ± 0.4	1.0 ± 0.5	−0.3 ± 0.4
Fasting plasma glucose (mmol/l)[a]	5.5 ± 1.0	−0.3 ± 0.8	5.9 ± 1.6	−0.7 ± 1.1*	5.2 ± 0.5	−0.3 ± 0.4	5.3 ± 0.8	−0.4 ± 0.6
Number of CHD risk factors, number per person	2.0 ± 1.0	−1.1 ± 0.9	1.6 ± 1.2	−0.9 ± 1.0	0.7 ± 0.8	−0.3 ± 0.9	0.9 ± 1.1	−0.5 ± 1.0

IF: intra-abdominal fat; ASF: abdominal subcutaneous fat; CHD: coronary heart disease. *$P<0.05$, **$P<0.01$, ***$P<0.001$: significantly different from diet alone group. [a]Analyses were performed on log transformed values for the variables.

second month 82.9 ± 10.3% and last month 89.1 ± 11.2%). The frequency of walking was 5.6 ± 1.4 day/week with an average duration of 187 ± 130 min/week. The average heart rate during walking was 115 ± 12 beats/min, which corresponds to 46.5 ± 4.4% of VO_{2max}. The mean energy expenditures during the walking and the bench stepping exercise were calculated as 849 ± 354 kcal/week and 1166 ± 130 kcal/week, respectively.

No difference was observed in intakes of total energy, proteins, fat and carbohydrates between the DA and DE groups either at baseline or during weight loss (Table 1). Table 2 shows subjects' measurement variables at baseline, and changes in the variables by treatment group and obesity phenotype. We compared these changes between treatment groups and obesity phenotype by considering the subjects as four subgroups: IF obesity in DA group, IF obesity in DE group, ASF obesity in DA group and ASF obesity in DE group. For the IF obesity phenotype, decreases in body weight, body mass index, percentage fat mass and fat mass were significantly greater in the DE group than in the DA group ($P<0.001$). Abdominal fat areas ($P<0.01$), and diastolic blood pressure and fasting plasma glucose ($P<0.05$) were also reduced to a much greater extent in the DE group than in the DA group. Furthermore, subjects in the DE group had a larger increase in VO_{2max} than subjects in the DA group. For the ASF obesity phenotype, no significant difference was found between the two treatment groups with the exception of VO_{2max}.

Figure 1 shows the comparison of fat mass change among the four subgroups. Values were adjusted for age, menopausal status, baseline fat mass and change in body weight.

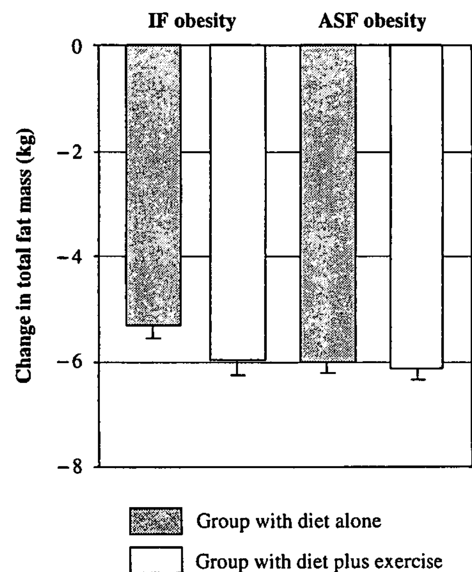

Figure 1 Comparisons of fat mass change between groups with diet alone and diet plus exercise. Values were adjusted for age, pre- or postmenopausal status, baseline total fat mass and change in body weight. IF = intra-abdominal fat, ASF = abdominal subcutaneous fat.

No difference was found in reduction of total fat among the four subgroups (−5.3 ± 0.3 kg IF/DA, −6.0 ± 0.2 kg IF/DE, −6.0 ± 0.3 kg ASF/DA and −6.1 ± 0.2 kg ASF/DE).

We also compared changes in IF areas among the four subgroups after adjusting for age, menopausal status, and changes in body weight and total fat mass (Figure 2). The IF

reductions were significantly greater for subjects classified with IF obesity in both the DA ($-37.8\pm3.4\,cm^2$, $P<0.05$) and DE ($-49.3\pm2.6\,cm^2$, $P<0.0001$) groups compared to subjects with ASF obesity (DA $-24.0\pm3.6\,cm^2$ and DE $-21.3\pm2.5\,cm^2$). Furthermore, for subjects with IF obesity, reductions in the IF area were significantly ($P<0.01$) greater in the DE group than in the DA group. On the other hand, for subjects with ASF obesity, there was no difference in IF area reduction between the DA and DE groups. We next compared changes in ASF areas among the four subgroups after adjusting for selected variables (Figure 3). There were significantly greater reductions in the ASF area of subjects with the ASF obesity phenotype in both the DA ($-70.6\pm5.6\,cm^2$, $P<0.05$) and DE ($-76.3\pm3.9\,cm^2$, $P<0.001$) groups, compared to subjects with the IF obesity phenotype (DA $-53.7\pm5.3\,cm^2$ and DE $-56.3\pm4.0\,cm^2$). However, there was no difference in the reduction of the ASF area between the DA and DE groups.

We quantified the independent contributions to the variances in the IF and ASF areas (Table 3). For the IF obesity phenotype, the reduction in IF area had a significant relationship to baseline IF area ($P<0.0001$), treatment ($P=0.0001$) and pre- or postmenopausal status ($P=0.0024$), and the reduction in ASF area was significantly related only to total fat mass rduction ($P<0.0001$). For the ASF obesity phenotype, reduction in IF area was related to baseline IF area ($P<0.0001$) and total fat mass reduction ($P=0.0018$), and the reduction in ASF area had a relationship to total fat mass reduction ($P<0.0001$) and baseline ASF area ($P=0.023$).

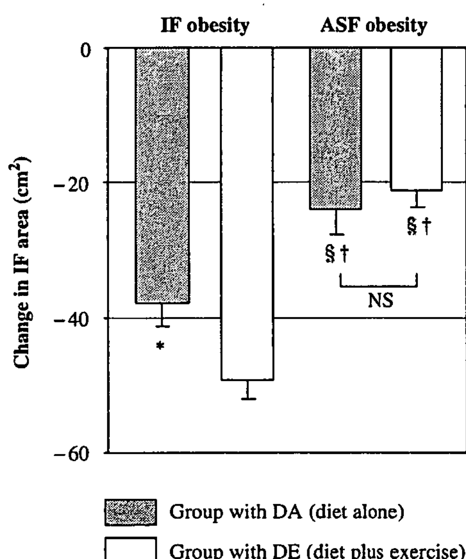

Figure 2 Comparisons of IF areas between groups with diet alone and diet plus exercise. Values were adjusted for age, pre- or postmenopausal status and changes in body weight and total fat mass. IF = intra-abdominal fat, ASF = abdominal subcutaneous fat. *$P<0.01$, §$P<0.0001$: significant difference from DE group with IF obesity. †$P<0.005$: significant difference from DA group with IF obesity.

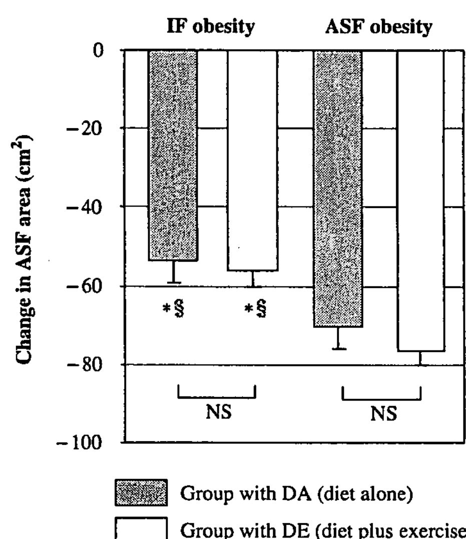

Figure 3 Comparisons of ASF areas between groups with diet alone and diet plus exercise. Values were adjusted for age, pre- or postmenopausal status, baseline ASF area and changes in body weight and total fat mass. IF = intra-abdominal fat, ASF = abdominal subcutaneous fat. *$P<0.001$: significant difference from DE group with ASF obesity. §$P<0.05$: significant difference from DA group with ASF obesity.

Table 3 Results of multiple regression analysis

Intra-abdominal fat obesity						*Abdominal subcutaneous fat obesity*					
Dependent variable	*Independent variable*	*Beta*	*F value*	*P-value*	*Model* R^2 (%)	*Dependent variable*	*Independent variable*	*Beta*	*F value*	*P-value*	*Model* R^2 (%)
ΔIF area	Baseline IF area	0.37	18.1	<0.0001	14.7	ΔIF area	Baseline IF area	0.39	55.1	<0.0001	35.5
	Treatment	−17.9	16.2	0.0001	26.2		Δtotal fat mass	1.7	10.3	0.0018	41.6
	Menstruation	−13.1	9.7	0.0024	32.5						
ΔASF area	Δtotal fat mass	6.0	40.9	<0.0001	28.0	ΔASF area	Δtotal fat mass	9.8	54.4	<0.0001	35.2
							Baseline ASF area	0.13	5.3	0.023	38.5

IF: intra-abdominal fat; ASF: abdominal subcutaneous fat. Δ: reduction. Treatment: 1 = diet alone; 2 = diet plus exercise; menstruation 0 = postmenopause, 1 = premenopause.

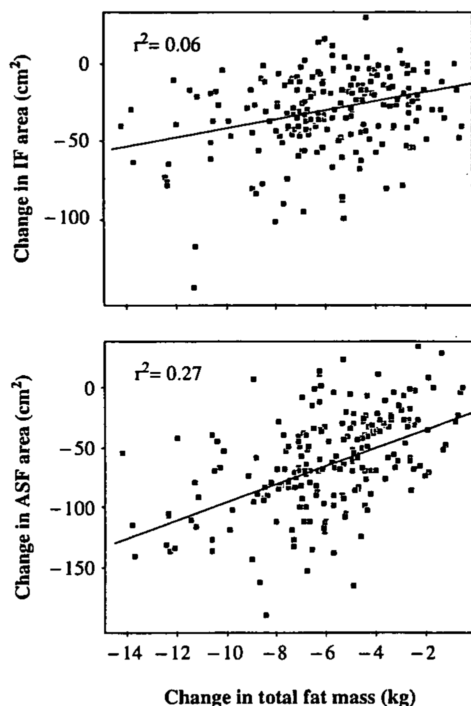

Figure 4 Relationships of fat mass change with changes in IF area and SF area. IF = intra-abdominal fat, ASF = abdominal subcutaneous fat.

Figure 4 shows the relationships between total fat mass change and changes in IF area and ASF area. The correlation coefficient between changes in ASF area and total fat mass ($r = 0.52$, $P < 0.0001$) was significantly larger than that between changes in IF area and total fat mass ($r = 0.24$, $P < 0.01$).

Discussion

Subjects with IF obesity had greater reductions in IF areas compared to subjects with ASF obesity (Figure 2), suggesting that obesity phenotype modifies IF reduction in response to weight loss. Obesity phenotype also modified ASF reduction in response to weight loss. Moreover, our data revealed that for individuals with IF obesity, the efficacy of adding aerobic exercise on reducing IF is more prominent ($-49.3\,cm^2/-37.8\,cm^2 = 1.3$ times) compared with DA.

Irwin *et al*[34] reported that an increase in duration (min/week) of physical activity had a significant association with the reduction of total fat, and a review by Ross and Janssen[16] suggested that an increase (≥ 1500 kcal/week) in physical activity for a short period (≤ 16 week) was associated with a reduction in total fat mass in a dose–response manner. Our study period was 14 weeks and the estimated energy expenditure during exercise was about 2000 kcal/week, which met the above criteria for reducing body fat. For the IF obesity phenotype (Table 2), reduction in total fat mass was greater in the DE group than in the DA group, which was attributable to the fact that weight loss was greater in the DE group than in the DA group. These results may simply reflect the difference between the negative energy balance of the two treatment groups for the IF obesity phenotype (DA −6069 kcal/week and DE −8084 kcal/week), and the significant difference in total fat mass change between the DA and DE groups did disappear when adjusted for the selected variables including body weight change (Figure 1). This means that total fat reduction per unit of negative energy balance by diet was equivalent to that by exercise training. That is, efficacy of exercise training on total fat reduction was equivalent to that of diet. Therefore, our data suggest that, with a strictly supervised weight loss program, exercise training could have a positive effect on reducing total fat and body weight.

Ross and Janssen[16] pointed out that baseline IF levels (obesity phenotype) may be associated with IF reduction in response to exercise training. Despres *et al*[25] found no significant reduction in IF area during a 14-month exercise training program with weight loss (−3.7 kg) in obese women having an initial mean IF area of 124.7 cm^2. Schwartz *et al*[22] reported that young men, whose initial mean IF area was 66.3 cm^2, had a slight reduction (−11.5 cm^2) in IF area during a 6-month endurance training program without weight loss, but older men, whose initial mean IF area was 144.5 cm^2, had a large reduction (−35.5 cm^2). Mourier *et al*[20] also found that patients with NIDDM (mean IF area 156.1 cm^2) had a significant reduction (−75.4 cm^2) in IF area during a 2-month endurance training program without weight loss. These studies seem to support Ross's assertion that obesity phenotype may be related to IF reduction secondary to exercise. Interestingly, our data (Figure 2) also indicate that reductions in IF areas were greater for the IF obesity phenotype (baseline levels of mean IF areas were DA 148 cm^2 and DE 135 cm^2), particularly in the DE group, than for the ASF obesity phenotype (DA 68 cm^2 and DE 69 cm^2). In addition, our data also revealed that obesity phenotype modifies ASF reduction in response to weight loss; however, no significant difference was observed in baseline ASF areas between IF obesity (DA 266 cm^2 and DE 289 cm^2) and ASF obesity (DA 264 cm^2 and DE 246 cm^2). Since reductions in total abdominal fat areas were similar among the subgroups with the exception of IF obesity in the DE group, the IF reductions and ASF reductions may be compensating each other. It is still unclear whether a threshold exists below which the IF and ASF mobilization in the weight loss response is markedly reduced.

There is insufficient evidence to determine whether an increase in physical activity is associated with a corresponding reduction in abdominal obesity in a dose–response manner.[26] According to a review by Ross and Janssen,[16] only six studies, including three randomized, controlled studies[20,21,24] and three nonrandomized studies,[22,23,25] considered effects of exercise training on abdominal fat area

measured by computed tomography scan or magnetic resonance imaging. From these studies, Ross and Janssen concluded that, although physical activity was associated with a reduction in IF area, it is not possible to establish a dose–response relationship.[16] However, sample sizes of these previous studies might be too small to provide conclusive evidence that exercise training actually reduces IF. In the present study, sample size required to observe a significant difference in IF area and ASF area between the groups with the DA and DE of the IF obesity was calculated when a significance level of 0.05 (α) and 80% of the power (β) of the test were used. The sample sizes were 68 and 57 for IF area and ASF area, respectively, which are less than our sample size (sum total number of IF obesity = 105). This suggests that these differences between the groups with the DA and DE were statistically relevant in the IF obesity. Therefore, it is hardly for our results to be concomitant with a type II error. Therefore, our data may provide evidence that efficacy of exercise training for reducing IF may exceed by 1.3 times that of DA only for subjects with the IF obesity phenotype; the specific effect of exercise was modified by obesity phenotype.

The reason as to why this effect was found only with IF obesity is unclear, but it may be partly explained by the data in Figure 4. This figure indicates that, although the reduction in ASF area is strongly associated with reduction in total fat and corresponds to a negative energy balance, the reduction in IF area is also associated with other factors, one of which may be an increase in physical activity through exercise training. Shimomura *et al*[27] found that plasma lipid intake and triglyceride synthesis in rats were reduced to a lesser extent in IF tissue than in ASF tissue in response to endurance training, resulting in a more prominent reduction of IF compared to ASF. Riechman *et al*[35] also reported that an association between daily physical activity and IF reduction was more prominent than the association between daily physical activity and ASF reduction. As we mentioned, however, detailed mechanisms that the specific exercise effect was modified by obesity phenotype is still unclear. To demonstrate the specific efficacy of exercise training, further, strictly controlled studies on associations between exercise training and IF reduction looking at obesity phenotype are needed.

There are some limitations of this study. First, there was a wide age range of subjects (21–66 y) including both pre- and postmenopausal women. Menopausal status and estrogen levels may independently influence total and abdominal fat accumulation. Second, activity of daily living with the exception of exercise training was not controlled. Hence, precise total energy balance could not be calculated. These factors might partly preclude our definitive conclusions. Third, to increase subject adherence to the weight loss programs during the intervention period, subjects were not randomized to the treatments. However, no difference was found in any variables between the DA and DE groups at baseline, which is independent of obesity phenotype (see Table 2). This suggests there was little, if any, influence on the measurement variables by assigning rather than randomizing subjects.

In conclusion, aerobic exercise training in conjunction to a controlled diet can have positive effects on reducing both total and abdominal fat under a strictly supervised weight loss program, and for both obesity phenotype, reductions in IF area and ASF area were positively associated with baseline values. That is, obesity phenotype modifies abdominal fat reduction in response to weight loss. Moreover, our data raises the possibility that for individuals with IF obesity, adding aerobic exercise training to a dietary weight-reduction program further (1.3 times) reduces IF compared with DA even if weight reductions would be identical in both two treatments (DA *vs* DE).

Acknowledgements

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ADRB3遺伝子多型が減量抵抗性に及ぼす影響
—The SMART Study

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原 著

ADRB3遺伝子多型が減量抵抗性に及ぼす影響
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索引用語：ADRB3，遺伝子多型，減量，肥満

本研究の目的はβ_3アドレナリン受容体(ADRB3)遺伝子多型が減量抵抗性に及ぼす影響を検討することであった．3ヵ月間の減量プログラムに参加した女性の中から，遺伝子解析のための血液提供に同意した156名を対象とした．参加者は食事制限のみを行う(diet：D)群47名と，食事制限に加えて有酸素性運動を実践する(diet plus exercise：DE)群109名に分けられた．さらに，ADRB3遺伝子多型の解析結果から，変異のないD群TT型32名，変異のあるD群TC/CC型15名，同様に，DE群TT型65名，DE群TC/CC型44名に分けられた．本研究では，減量抵抗性を参加者の年齢と体重から算出した減量予想値と減量実測値の差と定義した．体重減少量は，D群TT型で最も小さく(8.1±2.3kg)，DE群TC/CC型で最も大きかった(9.2±2.9kg)が，介入方法および遺伝子型による主効果は有意ではなかった．また，減量抵抗性(減量予想値－減量実測値)は，D群TT型で最も大きく(2.2±3.3kg)，DE群TC/CC型で最も小さかった(0.1±2.8kg)が，介入方法による主効果(p=0.10)および遺伝子型による主効果(p=0.05)は統計学的有意水準に達しなかった．以上の結果から，ADRB3遺伝子の変異を有していても，減量抵抗性を示さないことが示唆された．

はじめに

肥満はさまざまな疾患との関連が強く[1,2]，先進国，発展途上国のいずれにおいても増え続けている[3,4]．減量は肥満者に健康利益をもたらす上で有効であることから[5]，数多くの医療機関，自治体等において肥満外来や減量教室が開かれている．しかし，どのような減量処方に対しても大きな個体差が認められることから，遺伝因子や環境因子が減量しにくさ(減量抵抗性)に影響を与えていると考えられる．

β_3アドレナリン受容体(ADRB3)は7回膜貫通型のG蛋白共役受容体であり，褐色脂肪組織および白色脂肪組織に多く存在する．ADRB3を介した刺激は，褐色脂肪組織においては熱産生を亢進し，白色脂肪組織においては脂肪分解を促進する[6]．このADRB3遺伝子において，第64番残基であるトリプトファンがアルギニンに変異する多型(TGG→CGG)が1995年に報告された[7~9]．この遺伝子変異を高率にもつピマインディアンは肥満と糖尿病を発症しやすく[7]，非糖尿病のフィンランド人で変異を持つ者は上半身肥満，高血圧，高インスリン血症になりやすい[8]．また，病的肥満のフランス白人においては，変異を持つと20歳以降の体重増加量が大きくなる[9]．これらの報告を受けて，ADRB3は肥満に関連する候補遺伝子の1つとして注目されるようになった．

ADRB3遺伝子多型が肥満のなりやすさだけでなく，減量抵抗性にも影響を与えるかどうかについては意見が分

表1 研究参加者の身体的特徴

	全体 (n=156)	食事群 (n=47)	食事群＋運動群 (n=109)	群間差
年齢（歳）	47.6±8.1	48.4±7.7	47.3±8.2	ns
身長（cm）	156.5±5.5	155.9±5.3	156.8±5.6	ns
体重（kg）	68.3±8.0	69.5±8.8	67.8±7.6	ns
BMI（kg/m^2）	27.9±3.1	28.6±3.2	27.6±3.1	ns

BMI：body mass index, ns：not significant.

表2 減量抵抗性の算出式

減量抵抗性(kg)＝減量予想値(kg)－減量実測値(kg)

$$減量予想値(kg)=\sum_{d=1}^{d=90}(a\cdot W_d\cdot b-1200)/7700$$

a：基礎代謝基準値 23.6(～29歳)，21.7(30～49歳)，20.7(50歳～)[20]
W_d：d日目の体重
b：生活活動強度指数 「やや低い」とみなして1.5を代入[20]
なお，エネルギー摂取量は1200kcal，脂肪1kgあたり7700kcal[21]とした．

かれている[10~19]．日本人肥満女性を対象とした検討では，変異を持つ者は変異を持たない者と比べて減量効果が小さいと報告されている[10~12]が，同様の検討を試みた李ら[13]の結果や，欧米人や韓国人を対象とした検討[14~19]では，両者に有意差はみられていない．このような結果の差が生じる原因の1つとして，各研究参加者の年齢や初期体重が異なり，体重減少量がその影響を受けていることが挙げられる．そこで，本研究では，個人の年齢および初期体重から算出される減量予想値と減量実測値の差を減量抵抗性と定義し，ADRB3遺伝子多型がこの減量抵抗性に及ぼす影響を検討することにした．

対象と方法

1．研究参加者

本研究の参加者は，3ヵ月間の食事制限または食事制限に運動実践を加えた減量プログラムの提供を受けた肥満女性または肥満傾向女性214名であり，その中から遺伝子解析のための血液提供に同意した156名をデータ解析の対象とした．研究参加者には，研究の目的や減量プログラムの内容，測定内容についての説明会を開催し，研究内容を十分に理解させた上で，研究参加への同意を得た．これらの研究実施手続きについては，筑波大学倫理委員会の承認を得た．

研究参加者156名のうち，食事制限のみのプログラムに参加した者(diet：D)は47名，食事制限に運動実践を加えたプログラムに参加した者(diet plus exercise：DE)は109名であった．本研究では，複数回の介入研究の結果をあわせたデータを用いていることから，D群とDE群をランダムに割り付けたとはいえない．しかしながら，各群の身体的特徴は**表1**に示したように群間差はなく，また，閉経者が含まれる割合にも有意差は認められなかった(D：36.2%, DE：28.4%)．

2．測定項目

測定項目は身長，体重，BMIであり，PCR-RFLP (polymerase chain reaction-restriction enzyme fragment length polymorphism)法を用いて，ADRB3遺伝子多型を分析した．また，**表2**の式を用いて減量抵抗性を算出した．

3．食事プログラム

食事プログラムの内容は，すべての参加者に共通したものである．本研究で指導した食事内容は1食あたり400 kcalを目標に，4群点数法[22]を用いて，栄養バランスのよい食事を摂取するように指導した．また，摂取エネルギー量を抑えながらも良好な栄養状態を維持する目的で，減量補助食品としてその顕著な効果が認められている[23~26]マイクロダイエット(サニーヘルス社)を1日1～2食併用した．1日の目標摂取エネルギーは1,200kcalであり，対象者には1食ごとの食事内容を記録させ，その記録をもとに摂取エネルギー量を確認し，管理栄養士が食習慣などについて指導した．

4．運動プログラム

運動プログラムの内容は，週3回の監視型運動プログラムと，週1回の監視型運動＋自宅での運動実践を組み合わせたプログラムに分けられる．週3回の監視型運動プログラムの内容は，ベンチステップエクササイズ[27,28]やペース(programmed aerobic/anaerobic/accommodating circuit exercise：PACE)[29]，レジスタンス運動などであり，それらを単独または組み合わせた形で提供した(主運動の時間は約45分間)．一方，週1回の監視型運動＋自宅での運動実践を組み合わせたプログラムの主運動はウォーキングであり，週1回の指導に加えて，できるだけ毎日，自宅付近で運動を実践するように指示した(1回あたり約30分間)．週3回の監視型運動の強度は主観的運動強度(rating of perceived exertion：RPE)で13(ややきつい)から17(とてもきつい)，ウォーキングの運動強度はRPE13(ややきつい)あたりであったことから，運動による消費エネルギーは1週間あたり1000kcal程度となる[30]．

5．統計解析

各項目の測定結果は平均値±標準偏差で表した．比率の差の検定にはχ^2検定を用い，相関関係についてはPear-

sonの積率相関係数を算出した．平均値の差を検定するためには，同一群内では対応のあるt検定を，独立した2群間では対応のないt検定を用いた．体重減少量または減量抵抗性の群間比較には介入方法×遺伝子型の二元配置分散分析，または初期体重の影響を共変量とした二元配置共分散分析を用いた．すべての統計解析にはSPSS 11.0Jを用い，統計学的有意水準を5%に設定した．

表3　各群における体重変化(単位：kg)

		減量前	減量後	体重減少量
D群	TT型(N=32)	70.8±8.9	62.7±8.7	8.1±2.3
	TC/CC型(N=15)	66.8±8.4	58.5±7.7	8.3±3.7
DE群	TT型(N=65)	67.8±7.8	59.1±7.2	8.6±2.9
	TC/CC型(N=44)	67.9±7.4	58.7±6.7	9.2±2.9

表4　各群における減量抵抗性(単位：kg)

	TT型	TC/CC型	計
D群	2.2±3.3	0.7±3.6	1.7±3.4
DE群	0.9±3.1	0.1±2.8	0.6±3.0
計	1.3±3.2	0.3±3.0	0.9±3.2

結　果

1．ADRB3遺伝子多型

研究参加者156名のうち，TGG(トリプトファン)をホモ型で有する者(TT型)が97名，TGG(トリプトファン)がCGG(アルギニン)に変異する多型をヘテロで有する者(TC型)は55名，ホモで有する者(CC型)は4名であった．本研究においては，TC型とCC型を合わせて変異型とみなした．TC/CC型が含まれる割合は，D群では32%(15名)，DE群では40%(44名)であり，両群間の比率に有意差はなかった．

2．各群における体重変化

D群およびDE群の体重変化を，TT型およびTC/CC型に分けて**表3**に示した．減量前の初期体重がD群のTT型とTC/CC型で差がみられたが有意ではなかった(P=0.15)．体重減少量に対して，介入方法×遺伝子型の二元配置分散分析を施したところ，介入方法の主効果(P=0.16)，遺伝子型の主効果(P=0.45)，両者の交互作用(P=0.71)，いずれも有意ではなかった．

3．減量予想値と減量実測値

研究参加者156名における減量前の年齢および体重を用いて算出した減量予想値は9.6±2.5kg(4.5～19.2kg)であったのに対し，減量実測値は8.6±2.9kg(1.2～19.7kg)であり有意差が認められた．また，減量抵抗性(減量予想値－減量実測値)は，減量前の初期体重とr=0.47の有意な相関関係があった(図)．

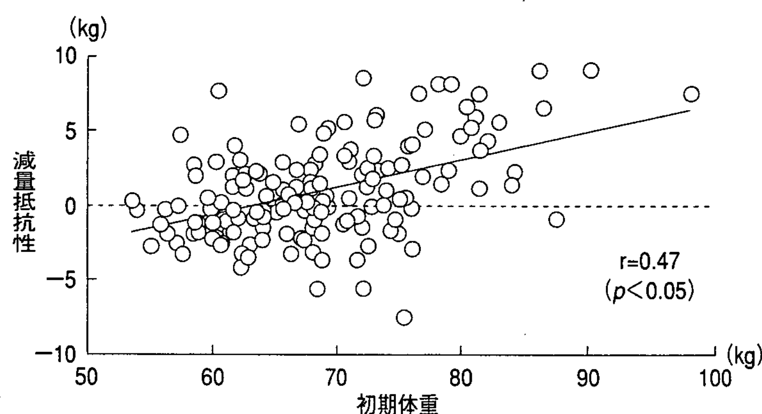

図　減量予想値と減量実測値との関係

4．各群における減量抵抗性

各群における減量抵抗性を検討したところ，D群TT型(2.2±3.3 kg)が最も大きく，DE群TC/CC型(0.1±2.8kg)が最も小さかった(**表4**)．二元配置の分散分析の結果，介入方法の主効果(p=0.10)，遺伝子型の主効果(p=0.05)，両者の相互作用(p=0.98)，いずれも有意ではなかった．また，減量前の初期体重で補正すると，介入方法の主効果はp=0.14，遺伝子型の主効果はp=0.13となった．

考　察

従来の減量介入研究は，対象となる集団に対し，複数の減量プログラムを提供し，その効果を検討してきた．しかし，どの減量プログラムに対しても効果は一様ではなく，大きな個体差が認められる．筆者らは，その個体差を肥満関連遺伝子多型，年齢および閉経の前後，形態，体組成，体脂肪分布，体力レベルの個人差などによって説明しようとするプロジェクトに2004年から着手している(The SMART Study：a study on Strategy for the MAde-to-order weight Reduction in Tsukuba)．本研究はそのプロジェクトの一環として，ADRB3遺伝子多型が体重減少量の個体差に及ぼす影響を検討した．

本研究の特長は，減量抵抗性を単に減量処方に対する体重減少量と定義するのではなく，個人の年齢と初期体重