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Low hydration status may be associated with insulin resistance and fat distribution: analysis of the Korea National Health and Nutrition Examination Survey (KNHANES) 2008–2010

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Abstract

We aimed to identify the association of hydration status with insulin resistance (IR) and body fat distribution. A total of 14 344 adults participated in the Korea National Health and Nutrition Examination Survey 2008–2010. We used urine specific gravity (USG) to indicate hydration status, and HOMA-IR (homoeostasis model assessment of IR) and trunk:leg fat ratio (TLR) as primary outcomes. In multivariate logistic regression, the OR per 0·01 increase in USG for high IR was 1·303 (95 % CI 1·185, 1·433; $P < 0·001$). In multivariate generalised additive model plots, increased USG showed a J-shaped association with logarithmic HOMA-IR, with the lowest Akaike's information criterion score of USG 1·030. Moreover, increased USG was independently associated with increased trunk fat, decreased leg fat and increased TLR. In mediation analysis, the proportion of mediation effects of USG on TLR via IR was 0·193 (95 % CI 0·132, 0·285; $P < 0·001$), while the proportion of mediation effects of USG on IR via TLR was 0·130 (95 % CI 0·086, 0·188; $P < 0·001$). Increased USG, a sign of low hydration status and presumably high vasopressin, was associated with IR and poor fat distribution. Direct effect of low hydration status may be more dominant than indirect effect via IR or fat distribution. Further studies are necessary to confirm our findings.

Key words: **Hydration: Urine specific gravity: Vasopressin: Insulin resistance: Body fat distribution**

Vasopressin (AVP), also known as antidiuretic hormone, is well known for its role in conserving water via V2 receptors expressed in the renal collecting ducts[1]. However, there are several unfamiliar actions of AVP beyond its antidiuretic effect. First, via its original antidiuretic action, increased AVP was associated with glomerular hyperfiltration[2,3]. We recently confirmed that 'sub-morbid dehydration', a condition in which AVP is presumably elevated, was associated with glomerular hyperfiltration in general Korean population[4].

Second, AVP influences glucose metabolism in different ways. AVP stimulates glycogenolysis and gluconeogenesis via V1a receptors expressed in the liver[5,6]. AVP stimulates the release of either glucagon or insulin, depending on extracellular glucose concentration, through the activation of V1b receptors in pancreatic islets[7]. AVP has also been shown to stimulate cortisol release through activation of the hypothalamus–pituitary–adrenal (H-P-A) axis, via V1b receptor expressed in the anterior pituitary, thus further influencing glucoregulatory mechanisms[5,6]. This unfamiliar role of AVP in glucose metabolism has been recently supported in several clinical studies showing that increased copeptin, the C-terminal part of the AVP precursor peptide[8], used as a surrogate marker of AVP, was associated with increased risk of insulin resistance (IR)[9–11], the metabolic syndrome[12–14] and diabetes[6,9,15–18].

Third, AVP may also affect fat metabolism. V1a receptor is expressed in both brown and white adipose tissues, while V1b receptor is expressed only in white adipose tissue[19]. It is suggested that activation of V1a receptor is associated with enhanced lipogenesis[19], while activation of V1b receptor is associated with enhanced lipolysis[20] along with enhanced IR in adipose tissue[21]. Unlike glucose metabolism, however, the potential effect of AVP on fat metabolism has rarely been evaluated in clinical studies[17].

Physiologically, a low hydration status is a major stimulus for the increase in AVP level[1]. Although hydration status can be evaluated in several ways[22], urine specific gravity (USG) is frequently used[23], particularly in large-scale populations[24]. Increased USG may indicate a low hydration condition and thus,

Abbreviations: AIC, Akaike's information criterion; BP, blood pressure; FG, fasting glucose; HOMA, homoeostasis model assessment; IR, insulin resistance; KNHANES, Korea National Health and Nutrition Examination Survey; TLR, trunk:leg fat ratio; USG, urine specific gravity; WC, waist circumference.

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presumably, elevated AVP[25]. Therefore, we hypothesised that a low hydration status or presumably elevated AVP status may be associated with increased risk of IR and poor fat distribution. To investigate our study hypothesis, we analysed the relationship of USG with IR and fat distribution in community-dwelling Korean adults, using data from the Korea National Health and Nutrition Examination Survey (KNHANES).

Methods

Participants

The KNHANES has been performed periodically since 1998 to assess the health and nutritional status of the civilian, non-institutionalised Korean population. Participants are being selected using proportional allocation and systematic sampling with multistage stratification. This study used data from the KNHANES 2008–2010. Of 36 188 candidates, 29 235 agreed to participate in the survey (participation rate, 80·8 %). The protocol comprised a health questionnaire survey, health examination and nutrition survey. Among the 29 235 participants, 7664 people whose age was <20 years were excluded. Of 21 571 adult participants, 5210 people with missing body fat measurements, eighty-seven with missing anthropometric measurements, 668 with missing fasting insulin and glucose levels, 885 with missing USG values and 377 with fasting time <8 h were further excluded. Therefore, this study included 14 344 participants.

Ethics statement

The study protocol complied with the Declaration of Helsinki, and full approval of the study was obtained from the Institutional Review Board of the Korea Centers for Disease Control (Institutional Review Board numbers: 2008-04EXP-01-C, 2009-01CON-03-2C and 2010-02CON-21-C). All data were fully anonymised before we accessed them. KNHANES participants provided informed written consent to have their data used in research.

Urine specific gravity, insulin resistance and body fat measurements

All the blood and urine samples were collected in the morning after overnight fast. Samples were transported and analysed at the central laboratory (Neodin Medical Institute). USG and proteinuria were measured with a dipstick method using the UriSys 2400 analyser (Roche), and analysing range was 1·000 to 1·050 for USG and – to 4+ for proteinuria.

The homoeostasis model assessment (HOMA) was used to calculate IR using the following equation: HOMA-IR = fasting insulin (μIU/ml) × fasting glucose (FG) (mmol/l)/22·5[26]. With the participant in the erect position, height was measured with a stadiometer (Seca 225; Seca) to the nearest 0·1 cm. Body weight was measured with the participant in a light gown with bare feet, with the use of digital scales (GL-6000-20; G-Tech) to the nearest 0·1 kg, and BMI was calculated by dividing the weight by the square of the height (kg/m^2). Waist circumference (WC) was measured with a tape measure (Seca 200; Seca) to the nearest 0·1 cm at the midpoint between the lower border of the rib cage and the highest point of the iliac crest of the participant.

Regional fats were measured using dual-energy X-ray absorptiometry with the QDR 4500A analyzer (Hologic Inc.) and calculated using Hologic Discovery software (version 3.1). The subregions of the head, arms, legs and trunk were delineated during the whole-body scan for assessment of regional fat amount. The trunk was defined using a horizontal line below the chin and vertical lines passing through the femoral neck comprising the right and left ribs, thoracic and lumbar spines and pelvic area. The arm regions were separated from the trunk at the levels of the shoulder joint; arm fat was defined as the sum of fat mass in both arms, and leg fat as the sum of fat mass in both legs. Trunk:leg fat ratio (TLR) was used as a fat distribution marker[27].

Other measurements

A standardised interview was conducted in the homes of the participants to collect information regarding demographic variables, medical history, medications used and other health-related variables. Blood pressure (BP) was measured three times in accordance with the standard protocol, and mean values of the three measurements were used as the representative BP. Total cholesterol, HDL-cholesterol, TAG, FG, and urinary Na levels were measured using the Hitachi 7600 Automatic Analyzer (Hitachi), and fasting insulin was measured using the 1470 WIZARD gamma counter analyzer (PerkinElmer). The estimated glomerular filtration rate was calculated using the Chronic Kidney Disease Epidemiology Collaboration equation[28]. Plain water and energy intake were estimated using the single day 24 h recall method.

Definitions

The following five metabolic disorders were defined based on the recommendations of the International Diabetes Federation[29]. Central obesity was defined as WC ≥ 90 cm for men and WC ≥ 80 cm for women. Raised BP was defined as systolic BP ≥ 130 mmHg, diastolic BP ≥ 85 mmHg, or treatment with antihypertensive drugs. Raised FG was defined as FG ≥ 5·6 mmol/l, treatment with insulin or oral anti-diabetic drugs, or a previous physician's diagnosis of type 2 diabetes. Raised TAG was defined as TAG ≥ 1·7 mmol/l. Reduced HDL-cholesterol level was defined as HDL-cholesterol < 1·03 mmol/l for men and HDL-cholesterol < 1·29 mmol/l for women, or having a specific treatment for dyslipidaemia.

Previous CVD was defined as a physician's diagnosis of cerebrovascular disease, angina or myocardial infarction. Alcohol drinking was defined as drinking alcoholic beverage more than twice a week. High-intensity exercise was defined as doing strenuous exercise (e.g. soccer, climbing) more than 3 d/week. Mid-intensity exercise was defined as doing moderate exercise (e.g. slow swimming, table tennis) more than 3 d/week. Proteinuria was defined as a protein level of 1+ or higher in the dipstick urinalysis. Furthermore, high IR was defined as a score in the highest quartile of the HOMA-IR (≥2·8).

Statistical analysis

For describing the baseline characteristics of the participants, we used sex-specific quartiles of USG because plasma vasopressin

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and urine concentration are known to be higher in men than women[30]. The first quartile of USG was <1·014 in women and <1·016 in men, the second quartile was 1·014–1·017 in women and 1·016–1·019 in men, the third quartile was 1·018–1·021 in women and 1·020–1·023 in men and the fourth quartile was ≥ 1·022 in women and ≥ 1·024 in men. The distributions of continuous variables were evaluated using histograms and Q–Q plots. The TAG, FG, fasting insulin levels and HOMA-IR score were not normally distributed. Normally distributed continuous variables were expressed as mean values and standard deviations, non-normally distributed continuous variables as medians (interquartile ranges (IQR)), and categorical variables as percentages. P_{trend} was analysed for normally distributed continuous variables using a linear term of one-way ANOVA, for non-normally distributed continuous variables using the Jonckheere–Terpstra test, and for categorical variables using a linear-by-linear association. Differences were analysed using the Bonferroni *post hoc* analysis of one-way ANOVA for normally distributed continuous variables, the Mann–Whitney *U* test for non-normally distributed continuous variables, and the χ^2 test for categorical variables. OR and 95 % CI of USG for high IR were analysed using logistic regression analysis. The relationship between USG and regional fat depots was analysed using linear regression analysis. On multivariate analysis, covariates were chosen based on clinical and statistical relevance. For the potential non-linear association, a multivariate generalised additive model for Gaussian distributions was adapted to visualise the associations between USG and logarithmic HOMA-IR using 'mgcv' package in R Statistics (version 3.03). We used Akaike's information criterion (AIC) as the primary measure of the model fit[19]. In the AIC analysis, lower scores within the data set indicated a better model fit, and the USG with the lowest AIC value for the association between USG and logarithmic HOMA-IR was assumed to be the threshold value. Mediation analysis was performed using 'mediation' package in R Statistics (version 3.03). Mediator and outcome were interchanged between logarithmic HOMA-IR and TLR, while treatment (USG) and pre-treatment covariates (age, sex, smoking and drinking status, suicide thought, previous CVD, proteinuria, estimated glomerular filtration rate, systolic and diastolic BP, WC, BMI, HDL-cholesterol, logarithmic TAG, lean body mass, arm and head fat, high- and mid-intensity exercise, plain water and energy intake, urine Na, and leucocytes) were fixed. We performed 2000 times simulation for the results. Average of all causal mediation effects and direct effects was presented. A *P* value of <0·05 was considered statistically significant. All analyses, unless otherwise specified, were performed using SPSS version 22 (release 2013; IBM Corp.).

Results

Among the 14 344 participants, 45·1 % were men and the mean values of age were 49·8 (SD 15·8) years. The mean values of BMI and WC were 23·7 (SD 3·3) kg/m^2 and 81·4 (SD 9·8) cm, respectively. The prevalence rates of central obesity, raised TAG, reduced HDL-cholesterol, raised BP and raised FG were 37·7, 29·3, 47·6, 41·4 and 29·1 %, respectively. The mean values of body fat mass were 9·15 (SD 3·49) kg in the trunk region, 5·32 (SD 1·90) kg in the leg region, 1·99 (SD 0·77) kg in the arm region and 0·94 (SD 0·14) kg in the head region. The mean values of HDL-cholesterol were 1·24 (SD 0·28) mmol/l, and the medians of TAG, glucose and insulin levels were 1·22 (IQR 0·82, 1·84) mmol/l, 5·16 (IQR 4·83, 5·61) mmol/l and 61·4 (IQR 48·6, 79·2) pmol/l, respectively.

Mean values of USG were 1·017 (SD 0·006) in women and 1·019 (SD 0·005) in men ($P < 0·001$). The baseline characteristics of the participants according to sex-specific USG quartiles are depicted in Table 1. The age of the participants decreased, and the proportion of men increased as the sex-specific USG quartile increased. With the increase in sex-specific USG quartile, the rate of current smoking increased. People in the higher sex-specific USG quartile tended to drink less plain water, took more energy content and performed high-intensity exercise more. As the sex-specific USG quartile increased, the rates of previous CVD, raised BP, central obesity, reduced HDL-cholesterol, raised TAG and raised FG decreased. Moreover, as the sex-specific USG quartile increased, the estimated glomerular filtration rate increased.

We analysed the relationship between USG and IR (Table 2). On univariate logistic regression analysis, per 0·01 increase in USG was associated with 1·130 times higher odds for high IR. This relationship between USG and high IR was independent from various confounders: the OR per 0·01 increase in USG for high IR was 1·303 (95 % CI 1·185, 1·432; $P < 0·001$). Furthermore, we identified a J-shaped association between USG and logarithmic HOMA-IR with the lowest AIC score of USG 1·030 (Fig. 1). We also explored the relationship between USG and four fat depots using linear regression analysis (Table 3). On multivariate analysis, increased USG was not associated with fat mass in the arm and head regions, but it was independently associated with increased trunk fat and decreased leg fat, resulting in an increased TLR.

We performed mediation analyses to identify the mutual relationship among USG, IR and TLR (Figs. 2 and 3). The average direct effect per 0·01 increase in USG on TLR was 0·040 (95 % CI 0·026, 0·053; $P < 0·001$), while average causal mediation effect per 0·01 increase in USG via IR was 0·010 (95 % CI 0·007, 0·012; $P < 0·001$), resulting in the proportion of mediation effects of 0·193 (95 % CI 0·132, 0·285; $P < 0·001$) as shown in Fig. 2. The average direct effect per 0·01 increase in USG on logarithmic HOMA-IR was 0·052 (95 % CI 0·037, 0·067; $P < 0·001$), while average causal mediation effect per 0·01 increase in USG via TLR was 0·008 (95 % CI 0·005, 0·010; $P < 0·001$), resulting in the proportion of mediation effects of 0·130 (95 % CI 0·086, 0·188; $P < 0·001$) as shown in Fig. 3.

Discussion

The most well-known function of AVP is antidiuresis via V2 receptor[1]. However, according to recent studies, AVP may have additional pleiotropic effects including AVP-induced glomerular hyperfiltration[2,3] and AVP-induced hyperglycaemia[5,6]. Theoretically, AVP may also suppress insulin sensitivity via V1b receptor[21], while increased AVP has antilipolytic effects via V1a receptor[19], suggesting that increased AVP may participate in IR and obesity. AVP is difficult to measure[8].

Table 1. Clinical characteristics of the study population according to sex-specific urine specific gravity (USG) quartiles§
(Mean values and standard deviations for continuous variables; numbers and percentages for categorical variables; medians and interquartile ranges)

	Sex-specific quartile of USG (*n* 14 344)								
	Q1 (*n* 3116)		Q2 (*n* 3738)		Q3 (*n* 3636)		Q4 (*n* 3854)		
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	P_{trend}
USG range in women	<1·014		1·014–1·017		1·018–1·021		≥1·022		
USG range in men	<1·016		1·016–1·019		1·020–1·023		≥1·024		
USG	1·011	0·003	1·016*	0·001	1·020*†	0·001	1·025*†‡	0·003	<0·001
Age (years)	52·9	15·8	53·8	15·4	50·3*†	15·0	42·9*†‡	14·7	<0·001
Men									
n	1288		1643		1829*†		1715*‡		<0·001
%	41·3		44·0		50·3		44·5		
Current smoking									
n	607		747		927*†		927*†		<0·001
%	19·6		20·1		25·6		24·2		
Alcohol drinking									
n	617		804		927*†		786‡		0·115
%	19·9		21·7		25·7		20·6		
Suicide thought									
n	579		681		553*†		580*†		<0·001
%	18·7		18·3		15·2		15·1		
High-intensity exercise									
n	479		588		629		723*†		<0·001
%	15·4		15·8		17·4		18·8		
Mid-intensity exercise									
n	835		929		1000		1037		0·324
%	26·9		25·0		27·6		27·0		
Previous CVD									
n	151		184		138		87*†‡		<0·001
%	4·9		4·9		3·8		2·3		
Raised BP									
n	1462		1791		1496*†		1193*†‡		<0·001
%	47·1		48·1		41·2		31·0		
Systolic BP (mmHg)	123·1	19·0	123·1	18·4	120·3*†	16·7	115·7*†‡	15·7	<0·001
Diastolic BP (mmHg)	77·7	11·2	77·9	10·9	77·5	10·4	76·1*†‡	10·6	<0·001
Central obesity									
n	1176		1495		1391		1342†‡		0·002
%	37·7		40·0		38·3		34·8		
Waist circumference (cm)	81·1	9·5	81·7	9·5	82·0*	9·6	80·6†‡	10·4	0·032
BMI (kg/m^2)	23·4	3·2	23·7	3·2	23·8*	3·3	23·7*	3·5	<0·001
Reduced HDL-cholesterol									
n	1634		1864		1686*†		1645*†‡		<0·001
%	52·5		50·0		46·4		42·7		
HDL-cholesterol (mmol/l)	1·2	0·3	1·2	0·3	1·2	0·3	1·3*†‡	0·3	<0·001
Raised TAG									
n	1016		1176		1111		899*†‡		<0·001
%	32·6		31·5		30·6		23·3		
TAG (mmol/l)									
Median	1·3		1·3		1·3		1·1*†‡		<0·001
Interquartile range	0·9–1·9		0·9–1·9		0·8–1·9		0·7–1·6		

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Table 1. (*Continued*)

	Sex-specific quartile of USG (*n* 14 344)								
	Q1 (*n* 3116)		Q2 (*n* 3738)		Q3 (*n* 3636)		Q4 (*n* 3854)		
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	P_{trend}
Raised FG									
n	944		1189		1062		984		<0·001
%	30·4		31·9		29·3		25·6		
FG (mmol/l)									
Median	5·2		5·2		5·2		5·1*†‡		<0·001
Interquartile range	4·8–5·6		4·9–5·7		4·8–5·6		4·8–5·5		
Fasting insulin (pmol/l)									
Median	60·4		61·7		61·4		61·9*		0·016
Interquartile range	48·4–77·0		49·2–79·1		48·1–79·4		48·5–80·5		
Estimated glomerular filtration rate (ml/min per 1·73 m^2)	93·6	18·0	92·6	17·2	95·6*†	15·4	100·9*†‡	15·2	<0·001
Proteinuria									
n	27		53		32		58		0·100
%	0·9		1·4		0·9		1·5		
Whole body mass (kg)	60·4	10·8	61·0	10·9	62·9*†	11·2	63·3*†	11·8	<0·001
Lean body mass (kg)	43·3	9·2	43·8	9·3	45·5*†	9·7	45·5*†	9·9	<0·001
Trunk fat (kg)	9·0	3·4	9·2	3·4	9·2	3·5	9·2	3·7	0·011
Leg fat (kg)	5·2	1·9	5·2	1·8	5·2	1·9	5·6*†‡	2·0	<0·001
Arm fat (kg)	1·98	0·74	1·98	0·76	1·97	0·75	2·03‡	0·8	0·003
Head fat (kg)	0·93	0·13	0·93	0·14	0·95*†	0·14	0·94*†	0·14	<0·001
Plain water intake (litres/d)	1·10	0·67	1·03*	0·64	1·01*	0·67	0·98*	0·65	<0·001
Energy intake (1000 kcal/d)‖	1·87	0·79	1·85	0·77	1·96*†	0·87	1·99*†	0·89	<0·001
Urine Na (mmol/l)	92·4	39·6	134·0*	43·6	149·1*†	51·1	142·5*†‡	53·5	<0·001
Leucocytes (×10^3/μl)	6·0	1·7	6·0	1·7	6·1	1·7	6·1	1·7	<0·001

BP, blood pressure; FG, fasting glucose.
P_{trend} was analysed for normally distributed continuous variables by a linear term of one-way ANOVA, for non-normally distributed continuous variables by Jonckheere–Terpstra tests, and for categorical variables by a linear-by-linear association. *, † and ‡ meant $P < 0·008$ when compared with Q1, Q2 and Q3, respectively, using Bonferroni *post hoc* analysis of one-way ANOVA for normally distributed continuous variables, Mann–Whitney *U* tests for non-normally distributed continuous variables and χ^2 tests for categorical variables.
§ Conversion factors from conventional units to SI units were ×6·945 for fasting insulin, ×0·0259 for HDL-cholesterol, ×0·0113 for TAG, ×0·0555 for FG.
‖ To convert kcal to kJ, multiply by 4·184.

Table 2. Association between urine specific gravity (USG) and high insulin resistance*
(Odds ratios and 95 % confidence intervals)

	Univariate (*n* 14 344)			Model 1 (*n* 14 342)			Model 2 (*n* 14 342)			Model 3 (*n* 12 484)		
	OR	95 % CI	*P*	OR	95 % CI	*P*	OR	95 % CI	*P*	OR	95 % CI	*P*
USG (per 0·01 increase)	1·130	1·057, 1·209	<0·001	1·153	1·067, 1·245	<0·001	1·215	1·120, 1·318	<0·001	1·303	1·185, 1·433	<0·001
Sex-specific USG quartile												
Q2 *v.* Q1	1·157	1·035, 1·294	0·010	1·117	0·988, 1·262	0·077	1·083	0·956, 1·227	0·210	1·173	1·019, 1·350	0·026
Q3 *v.* Q1	1·128	1·008, 1·263	0·036	1·081	0·954, 1·224	0·221	1·061	0·935, 1·205	0·360	1·218	1·050, 1·411	0·009
Q4 *v.* Q1	1·170	1·047, 1·307	0·006	1·300	1·147, 1·473	<0·001	1·287	1·132, 1·462	<0·001	1·404	1·209, 1·630	<0·001

* The first quartile (Q1) of USG was <1·014 in women and <1·016 in men, the second quartile (Q2) was 1·014–1·017 in women and 1·016–1·019 in men, the third quartile (Q3) was 1·018–1·021 in women and 1·020–1·023 in men, and the fourth quartile (Q4) was ≥ 1·022 in women and ≥ 1·024 in men. High insulin resistance was defined as highest quartile of homeostasis model assessment of insulin resistance (≥2·8). OR and 95 % CI were calculated using logistic regression analysis. In model 1, cardio-metabolic confounders (systolic and diastolic blood pressure, waist circumference, BMI, HDL-cholesterol and TAG) were entered as covariates. In model 2, body composition indices (lean body mass, trunk fat, leg fat, arm fat and head fat) along with variables in model 1 were entered as covariates. In model 3, other variables (age, sex, smoking and drinking status, suicide thought, previous CVD, proteinuria, estimated glomerular filtration rate, high- and mid-intensity exercise, plain water and energy intake, urine Na, and leucocytes) were entered as covariates.

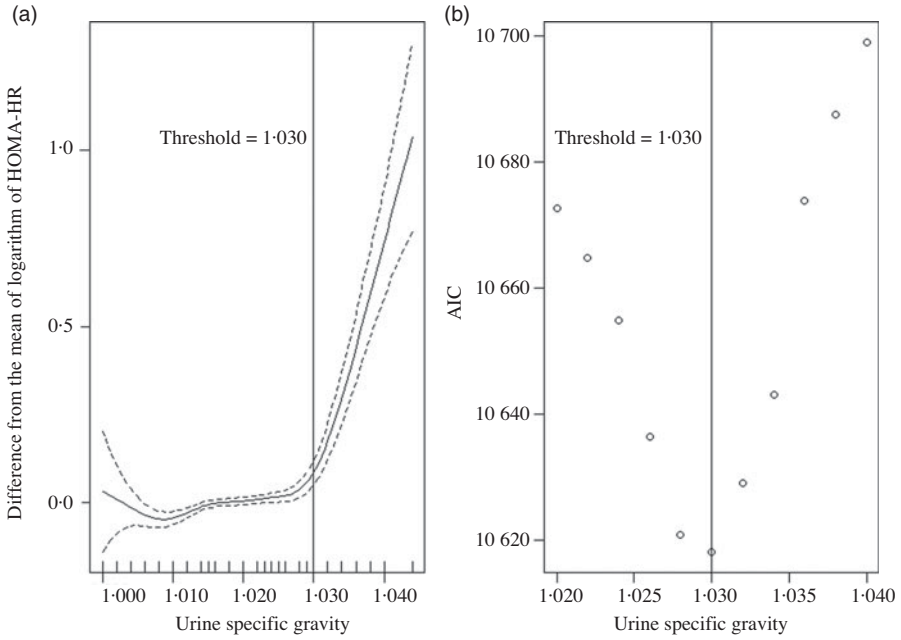

Fig. 1. Relationship between urine specific gravity and insulin resistance. The dashed line indicates 95 % CI for value of the smoothed logarithmic homoeostasis model assessment of insulin resistance (HOMA-IR) using multivariate generalised additive model analysis after adjusting for age, sex, smoking and drinking status, suicide thought, previous CVD, proteinuria, estimated glomerular filtration rate, systolic and diastolic blood pressure, waist circumference, BMI, HDL-cholesterol, TAG, lean body mass, trunk fat, leg fat, arm fat, head fat, high- and mid-intensity exercise, plain water and energy intake, and urine Na as covariates. AIC, Akaike's information criterion.

The measurement of copeptin, used as a surrogate marker of AVP[8], has not been incorporated in the ordinary practice. In this context, measuring the hydration status, based on USG, an index of urine concentration, may be a satisfactory phenotypic marker of AVP[25]. Among several hydration markers[22], USG is appropriate for large-scale populations[23,24]. Therefore, we hypothesised that high USG may be associated with increased risk of IR and poor fat distribution. In our large cohort, we identified that increased USG is independently associated with increased IR and poor fat distribution. We also found that the effect of USG on poor fat distribution is partially mediated by increased IR, while the hazard of USG on IR is partly mediated by poor fat distribution.

USG may be influenced by various conditions. If people consume more energy content, their waste production via urination may also increase, causing increased USG independent of water intake. Increased USG quartiles were associated with decreased cardiovascular risks, but this association was paralleled by a decrease in age. Because, in this study, mean USG was higher in men than women, in good agreement with the known sex difference in urine concentration[30], the effect of sex was also considered. In this way, the genuine effect of USG on IR was appropriately adjusted for the potential confounders. In this study, increased USG was independently associated with increased odds for high IR, even after adjusting for age, sex, habitual patterns, various cardiovascular risks, and plain water and energy intakes. Specifically, per 0·01 increase in USG was associated with 30·3 % increased odds for high IR in the multivariate logistic regression analysis. Using a generalised additive model plot, we also identified that increased USG showed a

Table 3. Relationship between urine specific gravity and four fat depots*
(*β* Values and 95 % confidence intervals)

	Exposure: per 0·01 unit increase of urine specific gravity					
	Univariate			Multivariate		
	β	95 % CI	*P*	*β*	95 % CI	*P*
Outcomes						
Trunk fat (kg)	–0·072	–0·173, 0·029	0·162	0·152	0·106, 0·198	<0·001
Leg fat (kg)	–0·066	–0·121, –0·011	0·018	–0·065	–0·098, –0·031	<0·001
Arm fat (kg)	–0·074	–0·096, –0·052	<0·001	0·002	–0·009, 0·013	0·671
Head fat (kg)	0·034	0·030, 0·037	<0·001	0·000	–0·004, 0·003	0·851
Trunk:leg fat ratio	0·005	–0·011, 0·021	0·527	0·040	0·027, 0·053	<0·001

* *β* Values and 95 % CI were analysed using linear regression analysis. In multivariate analysis, the covariates were logarithmic homeostasis model assessment of insulin resistance, systolic and diastolic blood pressure, waist circumference, BMI, logarithmic TAG, HDL-cholesterol, lean body mass, trunk fat, leg fat, arm fat, head fat, age, sex, smoking and drinking status, suicide thought, previous CVD, proteinuria, estimated glomerular filtration rate, high- and mid-intensity exercise, plain water and energy intake, urine Na, and leucocytes. When fat depots were chosen as an outcome, they were excluded from the model.

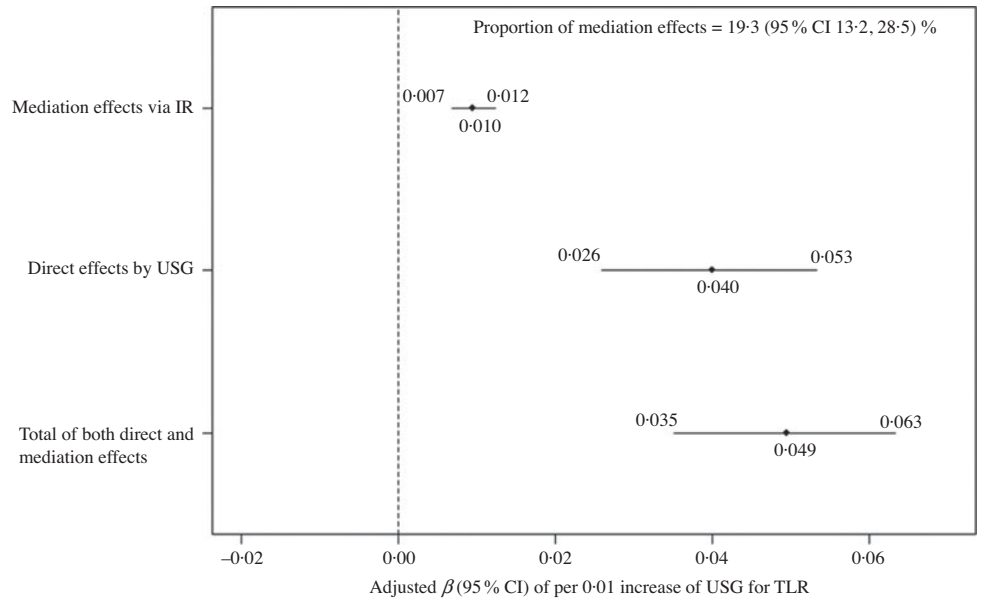

Fig. 2. Mediation analysis of insulin resistance (IR) for the relationship between urine specific gravity (USG) and fat distribution. IR was estimated by homoeostasis model assessment (HOMA). Outcome was trunk:leg fat ratio (TLR), treatment was USG, and mediator was logarithmic HOMA-IR. Overall effects of treatment on outcome were adjusted by mediator and pre-treatment covariates using multivariate linear regression analysis. Mediator was modelled by treatment and pre-treatment covariates using multivariate linear regression analysis.

J-shaped association with IR and the suggested threshold in the AIC analysis was USG ≥ 1·030. In our study population, the proportion of participants with USG ≥ 1·030 was only 2·2 %. Therefore, some may argue that although the hazard of low hydration status on IR may be statistically significant, the clinical significance of this low hydration may be limited. However, our results should be interpreted in the context of risk–benefit considerations. Several of the previous approaches to improve insulin sensitivity involved the use of various medications[10,19,31], which are not free from inadvertent side effects[9,32,33]. Although lifestyle modification with exercise is a must for people with diabetes, it is very difficult to achieve and maintain[34]. In contrast, low hydration status can be easily improved simply by drinking more water[35,36]. Therefore, the potential benefit of improved hydration status on glucose metabolism should be tested in future clinical trials. Actually, a recent study showed that, in adults with high copeptin levels, an increased hydration induced not only a significant decline in copeptin (as expected) but also a significant decline in glycaemia[37].

In the present study, we found that increased USG was associated with increased trunk fat and decreased leg fat, resulting in increased TLR, after adjusting for age, sex, habitual patterns, various cardiovascular risks and plain water and energy intakes. The relationship between USG and TLR was largely attributed to the consistent relationship between USG and leg fat. Although Chang *et al.* have suggested that increased urine osmolality was associated with a higher BMI after analysing 9528 US citizens[38], they did not analyse the effect of hydration status on regional adiposity any further. Therefore, our study is the first to demonstrate that hydration status is related to body fat distribution. The differential effects of low hydration status on body fat reminded us of different results of AVP on body fat according

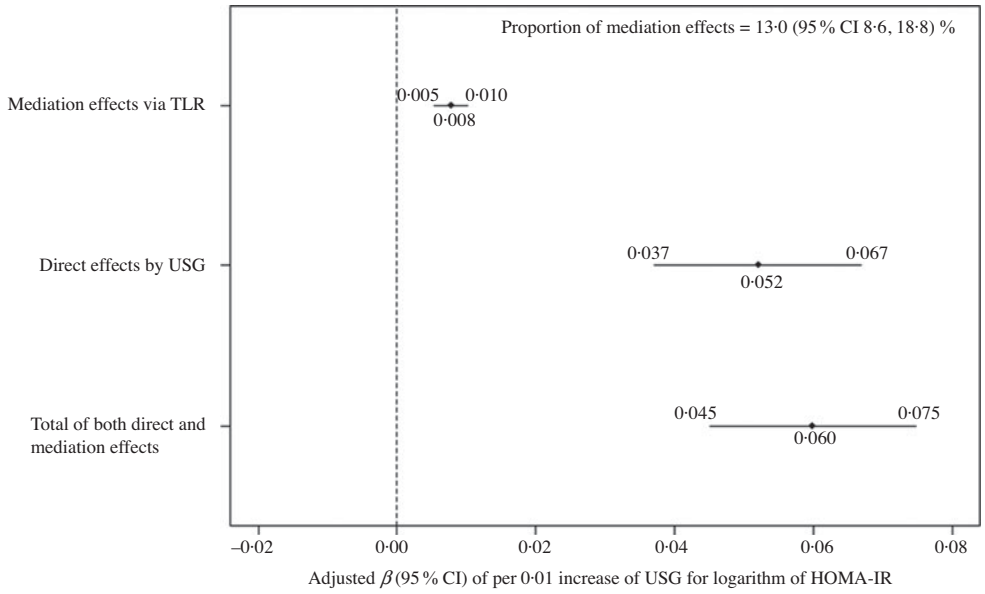

Fig. 3. Mediation analysis of fat distribution for the relationship between urine specific gravity (USG) and insulin resistance. Outcome was logarithmic homoeostasis model assessment of insulin resistance (HOMA-IR), treatment was USG, and mediator was trunk:leg fat ratio (TLR). Overall effects of treatment on outcome were adjusted by mediator and pre-treatment covariates using multivariate linear regression analysis. Mediator was modelled by treatment and pre-treatment covariates using multivariate linear regression analysis.

to the effects mediated by the different types of AVP receptors; the activation of V1a receptor resulted in fat accumulation, whereas activation of V1b receptor resulted in lipolysis[5]. Therefore, the influence of AVP on trunk fat may preferentially depend on the V1a receptor, whereas that on leg fat may preferentially depend on the V1b receptor. A possible differential expression of AVP receptors according to fat depots should be explored in the future experimental studies, and in clinical investigations.

AVP receptors are widely expressed in metabolic tissues, including adipose tissue, pancreas islet cells and the H-P-A axis[5]. Poor fat distribution is tightly associated with IR[39–42]. Therefore, it is highly suspected that the relationship of USG with poor fat distribution may be mediated via IR, and the effect of USG on IR may be mediated via poor fat distribution. In our mediation analysis, however, only 19·3 % of the effect of USG on fat distribution was mediated via IR, whereas only 13·0 % of the effect of USG on IR was mediated via poor fat distribution. These results may imply that direct effects of AVP via V1a and V1b receptors on target tissues are a more dominant pathway than the indirect effects via IR or poor fat distribution. Therefore, improving hydration status may be an easy and powerful strategy to improve public health by restoring insulin-glucose metabolism and fat distribution.

This study has several limitations. First, it is a cross-sectional study; therefore, a causal relationship cannot be established. However, the study hypotheses were constructed based on the known causality between AVP and metabolism of glucose and fat demonstrated in animal studies. We aimed to revise the focus on AVP, from its mechanism to its clinical context, to expand clinical utility. Second, USG was measured using the first morning urine sample after fast. USG from a single urine sample may have a potential error because it may not be representative of 24 h hydration status[43]. In addition, because of this spottiness, USG may simply mean tubular concentrating capacity, not usual hydration status. However, the concentration of the first morning urine is well correlated with that of 24 h urine[44]. Furthermore, in KNHANES, urine was always collected in morning after fast, suggesting urine may be standardised in sampling time. Therefore, although incomplete, USG in the first morning urine can be used as hydration marker, particularly in large-scale populations. Unfortunately, other hydration indices, including plasma and urine osmolality, bioimpedance analysis, and copeptin levels, were not measured because the KNHANES was not primarily designed to evaluate the hydration status. Finally, the generalisability of our results is limited because the study was conducted in a single country with a single ethnicity.

Besides these limitations, our study has several strengths. First, it is based on a well-defined very large population cohort (over 14 000 subjects) with a relatively uniform ethnic origin. Second, an extremely detailed evaluation of the metabolic phenotype was available, based on the measurement of a large number of variables. Third, same protocol to collect information and specimens was applied throughout study period. Furthermore, blood and urine samples were tested in the same central laboratory. Finally, this is the first study which suggested that hydration status, presumably AVP status, may affect body fat deposition differently depending on fat depots (trunk *v.* leg), which is a novel study subject to be confirmed by future animal and clinical studies.

In conclusion, this study revealed that a low hydration status, a presumably elevated AVP condition, was associated with increased IR and poor fat distribution. Direct effect of low hydration status may be more dominant than indirect effect via IR or fat distribution. Future studies are necessary to confirm our results.

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The authors declare that there are no conflicts of interest.

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