

BMJ Open

A significant association of daily physical activity with plasma B-type natriuretic peptide in patients with glucose intolerance: a cross-sectional study in National Center for Global Health and Medicine

Journal:	<i>BMJ Open</i>
Manuscript ID:	bmjopen-2014-006276
Article Type:	Research
Date Submitted by the Author:	04-Aug-2014
Complete List of Authors:	Hamasaki, Hidetaka; National Center for Global Health and Medicine Kohnodai Hospital, Department of Internal Medicine Yanai, Hidekatsu; National Center for Global Health and Medicine Kohnodai Hospital, Department of Internal Medicine Kakei, Masafumi; Jichi Medical University School of Medicine, Noda, Mitsuhiro; Center Hospital, National Center for Global Health and Medicine, Ezaki, Osamu; Showa Women's University,
Primary Subject Heading:	Diabetes and endocrinology
Secondary Subject Heading:	Diabetes and endocrinology
Keywords:	DIABETES & ENDOCRINOLOGY, SPORTS MEDICINE, PUBLIC HEALTH

SCHOLARONE™
Manuscripts

Only

Research**A significant association of daily physical activity with plasma B-type natriuretic peptide in patients with glucose intolerance: a cross-sectional study in National Center for Global Health and Medicine**

Hidetaka Hamasaki,^{1,2} Hidekatsu Yanai,¹ Masafumi Kakei,² Mitsuhiro Noda,³ Osamu Ezaki⁴

¹ Department of Internal Medicine, National Center for Global Health and Medicine Kohnodai Hospital, Chiba, Japan.

² Division of Complementary Medicine, First Department of General Medicine, Saitama Medical Center, Jichi Medical University School of Medicine, Saitama, Japan.

³ Department of Diabetes and Metabolic Medicine, Center Hospital, National Center for Global Health and Medicine, Tokyo, Japan.

⁴ Department of Human Health and Design, Faculty of Human Life and Environmental Sciences, Showa Women's University, Tokyo, Japan.

Correspondence to

Hidekatsu Yanai, MD, PhD, FACP, Department of Internal Medicine, National Center for Global Health and Medicine Kohnodai Hospital, 1-7-1 Kohnodai, Chiba 272-8516, Japan. E-mail: dyanai@hospk.ncgm.go.jp; Telephone: +81-47-372-3501; Fax: +81-47-372-1858

Keywords: B-type natriuretic peptide; glucose intolerance; insulin resistance; physical activity; type 2 diabetes

Word count: 2,237 words

ABSTRACT

Objectives: In spite of accumulating evidences suggesting an inverse association between insulin resistance and plasma B-type natriuretic peptide (BNP) levels, the effect of daily physical activity on plasma BNP in individuals with glucose intolerance remains unknown. We investigated the association of physical activity level (PAL) with plasma BNP in patients with impaired fasting glucose, impaired glucose tolerance and type 2 diabetes.

Design: Cross-sectional study.

Setting: Outpatients visiting National Center for Global Health and Medicine Kohnodai Hospital.

Participants: A total of 60 patients with glucose intolerance who did not take any hypoglycemic agents, cholesterol lowering agents and antihypertensive agents were recruited. Patients who were diagnosed as having heart failure and renal impairment, engaged in sports-like exercise and resistance training were excluded.

Primary outcome measures: PAL was objectively measured by a triaxial accelerometer. The association between PAL and plasma BNP was assessed by multiple regression analysis.

Results: PAL was positively correlated with plasma BNP levels ($r=0.328$, $p=0.011$).

1
2
3
4
5
6 PAL was still significantly correlated with plasma BNP levels after adjustment for age
7
8
9 ($\beta=0.320$, $p=0.016$), and adjustment for age and BMI ($\beta=0.312$, $p=0.019$). Plasma BNP
10
11
12 levels were inversely correlated with serum insulin levels ($r=-0.287$, $p=0.026$) and
13
14 homeostasis model assessment-estimated insulin resistance (HOMA-IR) ($r=-0.294$,
15
16 $p=0.023$). Serum insulin levels (mean \pm SD, 8.1 ± 6.4 $\mu\text{U/ml}$) and HOMA-IR ($2.4 \pm$
17
18 1.9) in high-BNP group were significantly lower than those (11.2 ± 7.4 $\mu\text{U/ml}$ and $3.7 \pm$
19
20 3.0 , respectively) in low-BNP group.
21
22
23
24

25
26 **Conclusions:** Our findings propose the possibility that plasma BNP may be increased
27
28
29 by daily physical activity and BNP is beneficially associated with insulin resistance.
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

Strengths and limitations of this study

- This study provides novel data: objectively measured light-intensity daily physical activity using a triaxial accelerometer is positively associated with plasma BNP levels in patients with glucose intolerance.
- Our study evaluated precisely an association between daily physical activity and plasma BNP levels, by recruiting drug-naive patients who were not engaged in sports-like exercise.
- Previous studies reported an inverse association between plasma BNP levels and the markers for insulin resistance in healthy subjects. To our knowledge, our study is the first to show a beneficial association between plasma BNP levels and insulin resistance in patients with glucose intolerance.
- The limitations are the small sample size and the cross-sectional design which does not allow us to establish any causal relationship.
- Even a triaxial accelerometer may lead to under or overestimation of energy expenditure. It can be denied that energy expenditure assessed by a triaxial accelerometer differ from the true amounts.

INTRODUCTION

B-type natriuretic peptide (BNP) belongs to the cardiac natriuretic peptide family which are released from the heart in response to pressure and volume overload.¹ Plasma BNP and N-terminal proBNP (Nt-proBNP) are increased with the severity of left ventricular dysfunction and/or hypertrophy, therefore, it has become a useful biomarker for diagnosis and prognosis of heart failure with or without type 2 diabetes.²⁻⁷

Plasma BNP levels have been reported to be inversely related to body mass index (BMI) and waist circumference in individuals without heart failure.⁸⁻⁹ Very recently, Olsen MH, et al. reported that Nt-proBNP was significantly lower in individuals with the metabolic syndrome as compared with those without the metabolic syndrome.¹⁰ In their study, Nt-proBNP levels were inversely correlated with BMI, waist circumference, serum cholesterol and triglyceride, plasma glucose and insulin, independently of age and gender.¹⁰ These results suggest that BNP is beneficially associated with obesity and obesity-related metabolic disorders.

Low physical activity and sedentary lifestyle induce the development of type 2 diabetes, and also deteriorate glucose control in patients with type 2 diabetes.¹¹⁻¹² Exercise such as bicycle and hand-grip exercise has been reported to acutely increase plasma BNP levels with exercise intensity, in healthy individuals.¹³⁻¹⁴ However, the

1
2
3
4
5
6 effect of daily physical activity on plasma BNP levels remain obscure. To our
7
8
9 knowledge, there were no previous studies that investigated the association between
10
11
12 daily physical activity and plasma BNP levels in patients with glucose intolerance.
13

14
15 Here, we studied the association of physical activity level (PAL) which was
16
17
18 evaluated objectively by using a triaxial accelerometer, with plasma BNP levels, in
19
20
21 patients with impaired fasting glucose (IFG), impaired glucose tolerance (IGT) and type
22
23
24 2 diabetes. Furthermore, we elucidated the correlations of plasma BNP levels to
25
26
27 metabolic parameters in patients with such glucose intolerance.
28
29
30
31

32 **MATERIAL AND METHODS**

33 34 35 **Study protocol and participants**

36
37
38 The study protocol was approved by the Medical Ethics Committee of National Center
39
40
41 for Global Health and Medicine (reference number NCGM-G-001212-00). We studied
42
43
44 60 participants (28 men and 32 women) who did not take any hypoglycemic agents,
45
46
47 cholesterol lowering agents and antihypertensive agents. To understand the effects of
48
49
50 daily physical activity such as non-exercise activity thermogenesis (NEAT)¹⁵ on BNP
51
52
53 and metabolic parameters, participants who were engaged in sports-like exercise and
54
55
56 resistance training were excluded. Participants with heart failure and renal impairment
57
58
59
60

1
2
3
4
5
6 were also excluded. The participants were diagnosed as having type 2 diabetes
7
8
9 according to diagnostic criteria set to serum levels of hemoglobin A1c (HbA1c) \geq
10
11
12 6.5 %, fasting plasma glucose (FPG) \geq 126 mg/dl, casual plasma glucose \geq 200
13
14 mg/dl and 2-h values in the 75 g oral glucose tolerance test (OGTT) \geq 200 mg/dl.¹⁶
15
16
17 The participants were defined as having IFG and IGT according to FPG levels 110
18
19 mg/dl to 125 mg/dl, or 2-h values in the OGTT of 140 mg/dl to 199 mg/dl.¹⁶
20
21
22
23
24
25

26 **Anthropometric and physiological measurements**

27
28
29 Height and weight were measured using a rigid stadiometer and scales (DP-7100PW,
30
31 Yamato Co., Ltd, Hyogo, Japan). Waist circumference around the navel was measured
32
33 with a metal anthropometric tape while participants are standing and breathing out. BMI
34
35 was calculated as the body weight in kilograms divided by the height in meters squared.
36
37
38 Blood pressure was measured with participants in a seated position using an automatic
39
40 sphygmomanometer (HEM-762, Omron Co., Ltd, Kyoto, Japan).
41
42
43
44
45
46
47
48

49 **Physical activity measurement**

50
51
52 Daily physical activity was measured using a triaxial accelerometer (Active Style Pro
53
54 HJA-350IT, Omron Co., Ltd, Kyoto, Japan), 74 x 46 x 34 mm and 60 g including
55
56
57
58
59
60

1
2
3
4
5
6 batteries. Participants studied wore the accelerometer on the left side of the waist.
7
8
9 Anteroposterior, mediolateral and vertical acceleration measurements were obtained
10
11 during each physical activity at a rate of 32 Hz to 12-bit accuracy. Each of three signals
12
13 from the triaxial accelerometer was passed through a high-pass filter with a cut-off
14
15 frequency of 0.7 Hz to remove the gravitational acceleration component. The ratios of
16
17 unfiltered to filtered total acceleration (TAU/TAF) and filtered vertical and horizontal
18
19 acceleration (VAF/HAF) were calculated to determine the cut-off value for the
20
21 classification of locomotive activities and non-locomotive activities including such as
22
23 household and occupational activities, which resulted in almost 100% accurate
24
25 demarcation for daily eleven different activities.¹⁷ Furthermore, metabolic equivalent
26
27 values (METs) determined by this triaxial accelerometer have been reported to be
28
29 closely correlated with METs calculated by using energy expenditure (EE) measured by
30
31 indirect calorimetry.^{17 18}
32
33
34
35
36
37
38
39
40
41
42
43

44 Participants studied wore the accelerometer on the left side of the waist for
45
46 consecutive 7 days, and physical activities were recorded. Participants were requested to
47
48 wear the accelerometer except under special circumstances such as sleeping, bathing
49
50 and during aquatic activities. Activity data were stored on a minute-by-minute basis and
51
52 were downloaded to a personal computer before analysis. We excluded days in which
53
54
55
56
57
58
59
60

1
2
3
4
5
6 participants did not wear the accelerometer for more than 8 hours from the data for
7
8
9 analysis.

10
11 Basal metabolic rate (BMR) was estimated from multiple regression equation
12 including age, sex, height and ideal body weight (IBW) as variables, the equation as
13 follows: $BMR \text{ (kcal/day)} = [(0.1283 + 0.0481 \times IBW \text{ (kg)} + 0.0234 \times \text{height (cm)} -$
14
15
16
17
18 $0.0138 \times \text{age (year)} - 0.5473 \times \text{sex coefficient (man: 1, woman:2)}] \times 293$.¹⁹ Total
19
20
21
22 energy expenditure (TEE) was calculated by manufactured regression equation using
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
METs assessed by the triaxial accelerometer.¹⁸ Physical activity level (PAL) was
calculated by the following equation. $PAL = TEE / BMR$.²⁰

Blood examination, BNP measurements

After a 12-h overnight fast, venous blood samples were taken from the antecubital vein
and collected into tubes. We measured FPG, HbA1c and serum insulin. FPG was
measured using an enzymatic method (Wako Pure Chemical Industries, Osaka, Japan).
Serum insulin and HbA1c were measured by automated enzyme-linked immunosorbent
assays (TOSOH, Tokyo, Japan). We used homeostasis model assessment-estimated
insulin resistance (HOMA-IR) as the marker for insulin resistance.²¹ Plasma BNP levels
were measured using specific immunoradiometric assay for human BNP (ARCHITECT

BNP-JP[®], ABBOTT JAPAN Co., Ltd, Tokyo, Japan).²²

Statistical analysis

Data were expressed as the mean \pm standard deviation (SD). Correlations among BNP, age, BMI, waist circumference, blood pressure, FPG, HbA1c, serum insulin levels, HOMA-IR and PAL were assessed by using the Pearson's correlation coefficient. Multiple regression analysis was performed to test the independent correlation of PAL with plasma BNP levels. We divided participants into high-BNP group (≥ 8.0 pg/ml) and low-BNP group (< 8.0 pg/ml) by the median values of BNP. Differences in serum insulin levels, HOMA-IR and PAL between high-BNP group and low-BNP group were analyzed by the Mann-Whitney U test. The statistical analyses were performed by using SPSS version 19 (IBM Co., Ltd, Chicago, USA). P value < 0.05 was considered to be statistically significant.

RESULTS

Table 1 presents the clinical characteristics of 60 participants (28 men and 32 women). 30 participants were diagnosed as having type 2 diabetes, 15 participants were with IFG and 15 participants were with IGT. The range of age in participants studied was 27-74

1
2
3
4
5
6 years old.
7
8

9 PAL evaluated by a triaxial accelerometer was significantly and positively
10 correlated with plasma BNP levels ($r=0.328$, $p=0.011$; **Figure 1**). Furthermore, PAL
11 was still significantly correlated with plasma BNP levels after adjustment for age
12 ($\beta=0.320$, $p=0.016$), and adjustment for age and BMI ($\beta=0.312$, $p=0.019$).
13
14
15
16
17
18
19

20 **Table 2** shows the correlations of plasma BNP levels to clinical and metabolic
21 parameters in participants. There were no significant correlations of plasma BNP levels
22 with age, BMI, waist circumference, systolic and diastolic blood pressure, FPG and
23 HbA1c. Plasma BNP levels were significantly and inversely correlated with serum
24 insulin levels and HOMA-IR.
25
26
27
28
29
30
31
32
33
34

35 To further understand the associations of plasma BNP levels with insulin
36 resistance, we examined the differences in serum insulin levels and HOMA-IR between
37 high-BNP group and low-BNP group (**Figure 2**). Serum insulin levels (mean \pm SD, 8.1
38 ± 6.4 U/ml) in high-BNP group were significantly lower than those (11.2 ± 7.4 U/ml) in
39 low-BNP group. HOMA-IR (2.4 ± 1.9) in high-BNP group were also significantly lower
40 than those (3.7 ± 3.0) in low-BNP group.
41
42
43
44
45
46
47
48
49
50
51
52
53
54

55 **DISCUSSION**

56
57
58
59
60

1
2
3
4
5
6 Recent cross-sectional studies reported that plasma BNP or NT-proBNP levels were
7
8 inversely associated with visceral fat,²³ BMI, waist circumference and serum insulin
9
10 levels.^{9 10} However, the effect of daily physical activity on plasma BNP levels remains
11
12 unknown. Previous cross-sectional studies have not ever been performed by using
13
14 individuals with glucose intolerance as our study participants. In the present study, we
15
16 examined the correlation of daily physical activity measured objectively by a triaxial
17
18 accelerometer with plasma BNP levels, in patients with prediabetes and early untreated
19
20 type 2 diabetes.
21
22
23
24
25
26
27
28

29 Present study demonstrate a significant and positive association between daily
30
31 physical activity and plasma BNP levels, and also an inverse association of plasma BNP
32
33 levels with serum insulin levels and HOMA-IR. Previous studies reported that exercise
34
35 such as bicycle and hand-grip exercise acutely increase plasma BNP levels with
36
37 exercise intensity in healthy individuals.^{13 14} However, the effect of daily physical
38
39 activity on plasma BNP levels remains unknown. It is noteworthy that we could observe
40
41 a significant effect of low-intensity daily physical activity, including walking, dishing
42
43 and washing clothes, defined as NEAT,¹⁵ on plasma BNP levels. Chainani-Wu N, et al.
44
45 previously conducted a nested prospective cohort study of a lifestyle intervention, to
46
47 understand the effects of lifestyle on BNP. They observed the change in BNP for 3
48
49
50
51
52
53
54
55
56
57
58
59
60

1
2
3
4
5
6 months was significantly associated with the change in exercise score, even after
7
8
9 controlling the percentage of change in BMI, which agreed with a significant and
10
11 positive correlation between PAL and plasma BNP levels after adjustment by age and
12
13 BMI in our study. At 3 months in their study, they found an inverse association of the
14
15 change in BNP with the changes in BMI and insulin,²⁴ suggesting a significant
16
17 association between BNP and insulin resistance.
18
19
20
21

22
23 An inverse association between natriuretic peptide and obesity and/or insulin
24
25 resistance has been established.^{8-10 23 25 26} However, controversy exists as to this
26
27 association. Lower natriuretic peptide levels in obese individuals has been reported to
28
29 be due to increased clearance by the natriuretic peptide receptors (NPR)-C abundantly
30
31 expressed in adipose tissue, and also due to decreased natriuretic peptide release from
32
33 the heart.²⁷⁻²⁹ BNP binds to NPR on adipose tissue and stimulate lipolysis,³⁰ regulates
34
35 adipose tissue by activation of proliferator-activated receptor gamma (PPAR γ) gene
36
37 expression³¹ and increases adiponectin secretion,³² which improves insulin resistance.
38
39
40
41
42
43
44
45

46
47 Evidences for the association of glucose intolerance with BNP are very limited. A
48
49 previous study reported that diabetes was significantly associated with low plasma BNP
50
51 levels (adjusted OR 1.51 (1.00 to 2.27) for men; adjusted OR 1.95 (1.26 to 3.02) for
52
53 women).⁸ However, the underlying mechanisms for low BNP levels in patients with
54
55
56
57
58
59
60

1
2
3
4
5
6 impaired glucose metabolism remain unknown. In present study, PAL was positively
7
8
9 correlated with plasma BNP levels, which was inversely correlated with the marker for
10
11
12 insulin resistance. Furthermore, serum insulin levels and HOMA-IR were significantly
13
14
15 lower in high-BNP group as compared with those in low-BNP group. However, neither
16
17
18 BMI and waist circumference were correlated with plasma BNP levels. Increased PAL
19
20
21 may elevate plasma BNP, which may ameliorate insulin resistance through various
22
23
24 mechanisms, such as reduced oxidative stress,³³ decreased systemic inflammation,³⁴
25
26
27 stimulated lipolysis,³⁰ activation of PPAR γ gene expression and increased adiponectin
28
29
30 secretion.^{31 32} However, the relationships among PAL, BNP and insulin resistance in
31
32
33 impaired glucose metabolism remain largely unknown. We should perform further
34
35
36 studies, preferably using a greater number of patients with glucose intolerance.

37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
There are several limitations that need to be considered when interpreting the
results of this study. This is a cross-sectional study, limiting inferences of causality and
its direction. Although we controlled for some confounding factors (age, sex, BMI,
engagement in sports-like exercise or resistance training, and medication), other factors
such as genetic variation were not taken into account. Furthermore, we studied only
patients with IFG, IGT and type 2 diabetes, therefore, our conclusions cannot apply to
healthy populations. A heterogeneous group of individuals with IFG, IGT and type 2

1
2
3
4
5
6 diabetes was examined. We could not mention that the associations between BNP and
7
8
9 physical activity or insulin resistance were the same in three groups. We will study these
10
11
12 associations, by using a great number of patients with each type of glucose intolerance,
13
14
15 in the future. The triaxial accelerometer is an extensively validated device for evaluating
16
17
18 physical activity under free living conditions,³⁵⁻³⁷ however, Leenders et al indicated that
19
20
21 the predictive equations based on the relationship between acceleration and energy
22
23
24 expenditure (EE) during locomotive movements led to under- and overestimation of
25
26
27 TEE.³⁸ It is possible that EE and PAL assessed by a triaxial accelerometer differ from
28
29
30 the true amounts of EE and PAL.

31
32 In conclusion, we found a significant and positive association between PAL and
33
34
35 plasma BNP levels in patients with prediabetes and early untreated type 2 diabetes.
36
37
38 Plasma BNP levels were inversely associated with insulin resistance. Our findings
39
40
41 propose the possibility that BNP could be increased by daily physical activity and
42
43
44 plasma BNP is beneficially associated with insulin resistance.
45
46
47
48

49 **Contributors**

50
51
52 The study was carried out in collaboration between all authors. HH, HY and OE
53
54
55 conceived and designed the study. HH and MN performed the study. HH, HY and MK
56
57
58
59
60

1
2
3
4
5
6 analyzed the data, interpreted the results and wrote the manuscript. HH and HY also
7
8
9 discussed analyses, interpretation, presentation and participated in drafting the
10
11
12 manuscript.

13 14 15 16 17 18 **Funding**

19
20 This study was supported by a grant from the National Center for Global Health and
21
22
23 Medicine (25-203).
24
25
26
27
28

29 **Competing interests**

30
31
32 The authors declare that there are no conflicts of interest.
33
34
35
36
37

38 **Ethics approval**

39
40 The study was approved by the Medical Ethics Committee of National Center for
41
42
43 Global Health and Medicine (reference number NCGM-G-001212-00).
44
45
46
47
48

49 **Provenance and peer review**

50
51
52 Not commissioned; externally peer reviewed.
53
54
55
56
57
58
59
60

Data sharing statement

No additional data are available.

REFERENCES

1. Gardner DG, Chen S, Glenn DJ, Grigsby CL. Molecular biology of the natriuretic peptide system: implications for physiology and hypertension. *Hypertension* 2007;49:419-26.
2. Maeda K, Tsutamoto T, Wada A, et al. High levels of plasma brain natriuretic peptide and interleukin-6 after optimized treatment for heart failure are independent risk factors for morbidity and mortality in patients with congestive heart failure. *J Am Coll Cardiol* 2000;36:1587-93.
3. Wang TJ, Larson MG, Levy D, et al. Plasma natriuretic peptide levels and the risk of cardiovascular events and death. *N Engl J Med* 2004;350:655-63.
4. Alehagen U, Lindstedt G, Levin LA, Dahlström U. Risk of cardiovascular death in elderly patients with possible heart failure. B-type natriuretic peptide (BNP) and the aminoterminal fragment of ProBNP (N-terminal proBNP) as prognostic indicators in a 6-year follow-up of a primary care population. *Int J Cardiol* 2005;100:125-33.
5. Kroon MH, van den Hurk K, Alsema M, et al. Prospective associations of B-type

1
2
3
4
5
6 natriuretic peptide with markers of left ventricular function in individuals with and
7
8
9 without type 2 diabetes: an 8-year follow-up of the Hoorn Study. *Diabetes Care*
10
11
12 2012;35:2510-14.

13
14
15 6. Olsen MH, Wachtell K, Tuxen C, et al. N-terminal pro-brain natriuretic peptide
16
17 predicts cardiovascular events in patients with hypertension and left ventricular
18
19 hypertrophy: a LIFE study. *J Hypertens* 2004;22:1597-604.
20
21
22

23
24 7. Schirmer H, Omland T. Circulating N-terminal pro-atrial natriuretic peptide is an
25
26 independent predictor of left ventricular hypertrophy in the general population. The
27
28 Tromsø Study. *Eur Heart J* 1999;20:755-63.
29
30
31

32
33 8. Wang TJ, Larson MG, Levy D, et al. Impact of obesity on plasma natriuretic peptide
34
35 levels. *Circulation* 2004;109:594-600.
36
37

38
39 9. Koizumi M, Watanabe H, Kaneko Y, et al. Impact of obesity on plasma B-type
40
41 natriuretic peptide levels in Japanese community-based subjects. *Heart Vessels* 2012;
42
43 27:287-294.
44
45

46
47 10. Olsen MH, Hansen TW, Christensen MK, et al. N-terminal pro brain natriuretic
48
49 peptide is inversely related to metabolic cardiovascular risk factors and the metabolic
50
51 syndrome. *Hypertension* 2005, 46:660-6.
52
53
54

55
56 11. Hamilton MT, Hamilton DG, Zderic TW. Role of low energy expenditure and sitting
57
58
59
60

1
2
3
4
5
6 in obesity, metabolic syndrome, type 2 diabetes, and cardiovascular disease. *Diabetes*
7
8
9 2007;56:2655-67.

10
11 12. Avery L, Flynn D, van Wersch A, Sniehotta FF, Trenell MI. Changing physical
12
13 activity behavior in type 2 diabetes: a systematic review and meta-analysis of behavioral
14
15 interventions. *Diabetes Care* 2012;35:2681-89.
16
17

18
19 20. Barletta G, Stefani L, Del Bene R, et al. Effects of exercise on natriuretic peptides
21
22 and cardiac function in man. *Int J Cardiol* 1998;65:217-25.
23
24

25
26 26. Nielsen HB, De Palo EF, Meneghetti M, Madsen PL, Ihlemann N, Secher NH.
27
28 Circulating immunoreactive proANP1-30 and proANP31-67 responses to acute exercise.
29
30
31
32 *Regul Pept* 2001;99:203-7.
33
34

35
36 36. Levine JA. Non-exercise activity thermogenesis (NEAT). *Nutr Rev* 2004;62:S82-97.
37

38
39 39. American Diabetes Association. Diagnosis and classification of diabetes mellitus.
40
41
42 *Diabetes Care* 2010;33:S62-9.
43

44
45 45. Oshima Y, Kawaguchi K, Tanaka S, et al. Classifying household and locomotive
46
47 activities using a triaxial accelerometer. *Gait Posture* 2010;31:370-4.
48

49
50 50. Ohkawara K, Oshima Y, Hikiyama Y, Ishikawa-Takata K, Tabata I, Tanaka S.
51
52 Real-time estimation of daily physical activity intensity by a triaxial accelerometer and
53
54 a gravity-removal classification algorithm. *Br J Nutr* 2011;105:1681-91.
55
56
57
58

- 1
2
3
4
5
6 19. Ganpule AA, Tanaka S, Ishikawa-Takata K, Tabata I. Interindividual variability in
7
8
9 sleeping metabolic rate in Japanese subjects. *Eur J Clin Nutr* 2007;61:1256-61.
10
11
12 20. Energy and protein requirements. Report of a joint FAO/WHO/UNU Expert
13
14 Consultation. *World Health Organ Tech Rep Ser* 1985;724:1-206.
15
16
17 21. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC.
18
19 Homeostasis model assessment: insulin resistance and beta-cell function from fasting
20
21 plasma glucose and insulin concentrations in man. *Diabetologia* 1985;28:412-419.
22
23
24 22. Mongia SK, La'ulu SL, Apple FS, Ler R, Murakami MM, Roberts WL. Performance
25
26 characteristics of the Architect brain natriuretic peptide (BNP) assay: a two site study.
27
28
29
30
31
32
33 *Clin Chim Acta* 2008;391:102-5.
34
35 23. Neeland IJ, Winders BR, Ayers CR, et al. Higher natriuretic peptide levels associate
36
37 with a favorable adipose tissue distribution profile. *J Am Coll Cardiol* 2013;62:752-60.
38
39
40 24. Chainani-Wu N, Weidner G, Purnell DM, et al. Relation of B-type natriuretic
41
42 peptide levels to body mass index after comprehensive lifestyle changes. *Am J Cardiol*
43
44
45
46
47 2010;105:1570-6.
48
49 25. Mehra MR, Uber PA, Park MH, et al. Obesity and suppressed B-type natriuretic
50
51 peptide levels in heart failure. *J Am Coll Cardiol* 2004;43:1590-5.
52
53
54
55 26. McCord J, Mundy BJ, Hudson MP, et al. Relationship between obesity and B-type
56
57
58
59
60

1
2
3
4
5
6 natriuretic peptide levels. *Arch Intern Med* 2004;164:2247-52.
7

8
9 27. Dessì-Fulgheri P, Sarzani R, Tamburrini P, et al. Plasma atrial natriuretic peptide and
10
11 natriuretic peptide receptor gene expression in adipose tissue of normotensive and
12
13 hypertensive obese patients. *J Hypertens* 1997;15:1695-9.
14
15

16
17 28. Das SR, Drazner MH, Dries DL, et al. Impact of body mass and body composition
18
19 on circulating levels of natriuretic peptides: results from the Dallas Heart Study.
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
Circulation 2005;112:2163-8.

29. Costello-Boerrigter LC, Burnett JC Jr. A new role for the natriuretic peptides:
metabolic regulators of the adipocyte. *J Am Coll Cardiol* 2009;53:2078-9.

30. Sengenès C, Berlan M, De Glisezinski I, Lafontan M, Galitzky J. Natriuretic
peptides: a new lipolytic pathway in human adipocytes. *FASEB J* 2000;14:1345-51.

31. Miyashita K, Itoh H, Tsujimoto H, et al. Natriuretic
peptides/cGMP/cGMP-dependent protein kinase cascades promote muscle
mitochondrial biogenesis and prevent obesity. *Diabetes* 2009;58:2880-92.

32. Tsukamoto O, Fujita M, Kato M, et al. Natriuretic peptides enhance the production
of adiponectin in human adipocytes and in patients with chronic heart failure. *J Am Coll
Cardiol* 2009;53:2070-7.

33. Rajagopalan S, Kurz S, Münzel T, et al. Angiotensin II-mediated hypertension in the

1
2
3
4
5
6 rat increases vascular superoxide production via membrane NADH/NADPH oxidase
7
8
9 activation. Contribution to alterations of vasomotor tone. *J Clin Invest*
10
11
12 1996;97:1916-23.

13
14 34. Moro C, Klimcakova E, Lolmède K, et al. Atrial natriuretic peptide inhibits the
15
16
17 production of adipokines and cytokines linked to inflammation and insulin resistance in
18
19
20 human subcutaneous adipose tissue. *Diabetologia* 2007;50:1038-47.

21
22
23 35. Plasqui G, Bonomi AG, Westerterp KR. Daily physical activity assessment with
24
25
26 accelerometers: new insights and validation studies. *Obes Rev* 2013;14:451-62.

27
28
29 36. Midorikawa T, Tanaka S, Kaneko K, et al. Evaluation of low-intensity physical
30
31
32 activity by triaxial accelerometry. *Obesity* 2007;15:3031-8.

33
34
35 37. Ekelund U, Sjöström M, Yngve A, et al. Physical activity assessed by activity
36
37
38 monitor and doubly labeled water in children. *Med Sci Sports Exerc* 2001;33:275-81.

39
40
41 38. Leenders NY, Sherman WM, Nagaraja HN. Energy expenditure estimated by
42
43
44 accelerometry and doubly labeled water: do they agree? *Med Sci Sports Exerc*
45
46
47 2006;38:2165-72.

1
2
3
4
5
6 (figure legends)
7
8

9 **Figure 1** Correlation of physical activity level and plasma BNP levels in 60 subjects.
10

11 r indicates correlation coefficient analyzed by the Pearson's correlation.
12
13

14
15
16
17 **Figure 2** Serum insulin levels and homeostatic model assessment-estimated insulin
18 resistance (HOMA-IR) in high-BNP group (n = 30) and low-BNP group (n = 30). Data
19 are mean values±SD. We divided subjects into high-BNP group (≥ 8.0 pg/ml) and
20 low-BNP group (< 8.0 pg/ml) by the median values of BNP. Differences in serum
21 insulin levels and HOMA-IR between high-BNP group and low-BNP group were
22 analyzed by the Mann-Whitney U test.
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

Table 1 Clinical and biochemical characteristics of subjects

n	60
Age (years)	54.7 ± 12.2
Height (cm)	161.3 ± 8.9
Weight (kg)	69.2 ± 16.1
BMI (kg/m ²)	26.5 ± 5.1
Waist circumference (cm)	92.4 ± 13.1
Systolic blood pressure (mmHg)	128.8 ± 17.6
Diastolic blood pressure (mmHg)	80.5 ± 12.5
Fasting plasma glucose (mg/dl)	122.9 ± 25.6
HbA1c (%)	6.7 ± 1.1
BNP (pg/ml)	14.0 ± 14.6
Serum insulin (μU/ml)	9.6 ± 6.9
HOMA-IR	3.02 ± 2.62

Data are expressed as the mean ± SD. BMI: body mass index, BNP: B-type natriuretic peptide, HOMA-IR: homeostasis model assessment-estimated insulin resistance,

Table 2 Correlations of plasma BNP levels to clinical and metabolic parameters

	correlation coefficient	<i>P</i> Value
Age (years)	0.245	0.060
BMI (kg/cm ²)	-0.184	0.159
Waist circumference (cm)	-0.217	0.096
Systolic blood pressure (mmHg)	0.103	0.436
Diastolic blood pressure (mmHg)	-0.020	0.883
Fasting plasma glucose (mg/dl)	-0.209	0.098
HbA1c (%)	-0.014	0.917
Serum insulin (μU/ml)	-0.287	0.026
HOMA-IR	-0.294	0.023

BMI: body mass index, BNP: B-type natriuretic peptide, HOMA-IR: homeostasis model assessment-estimated insulin resistance.

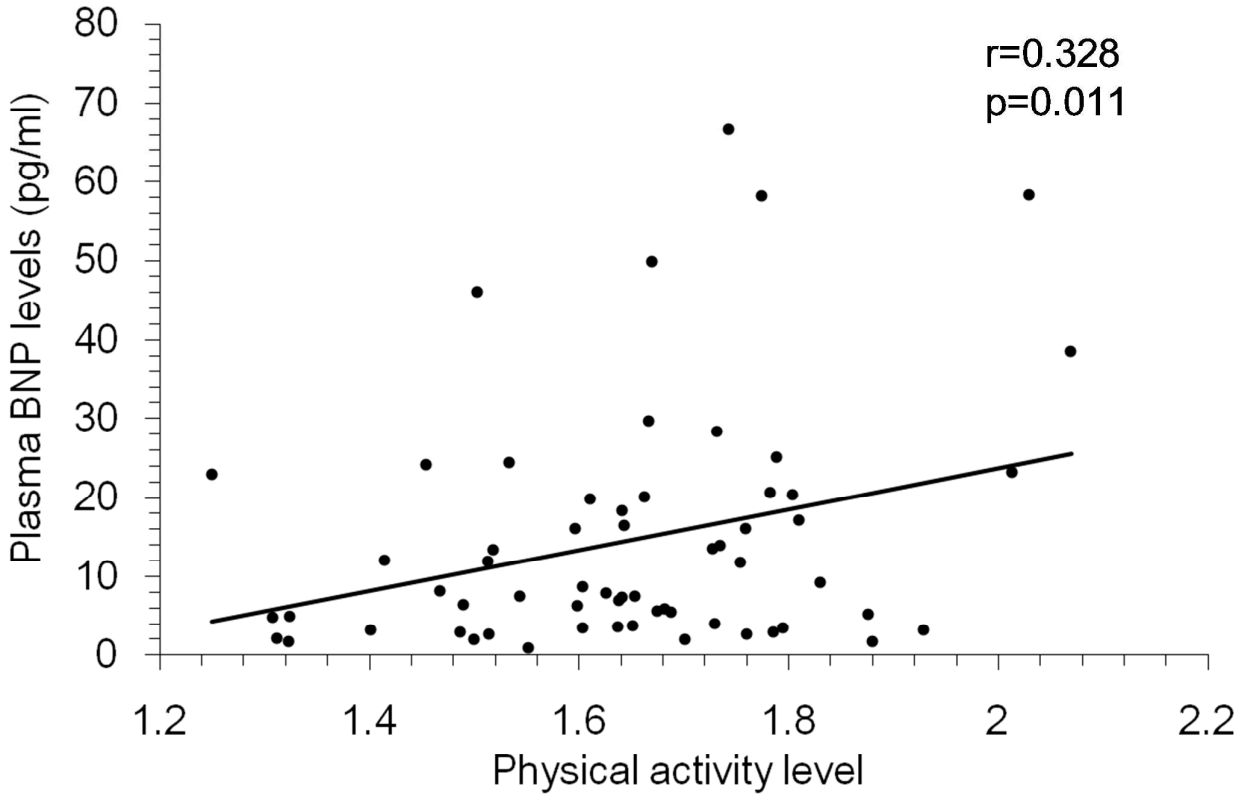


Figure 1

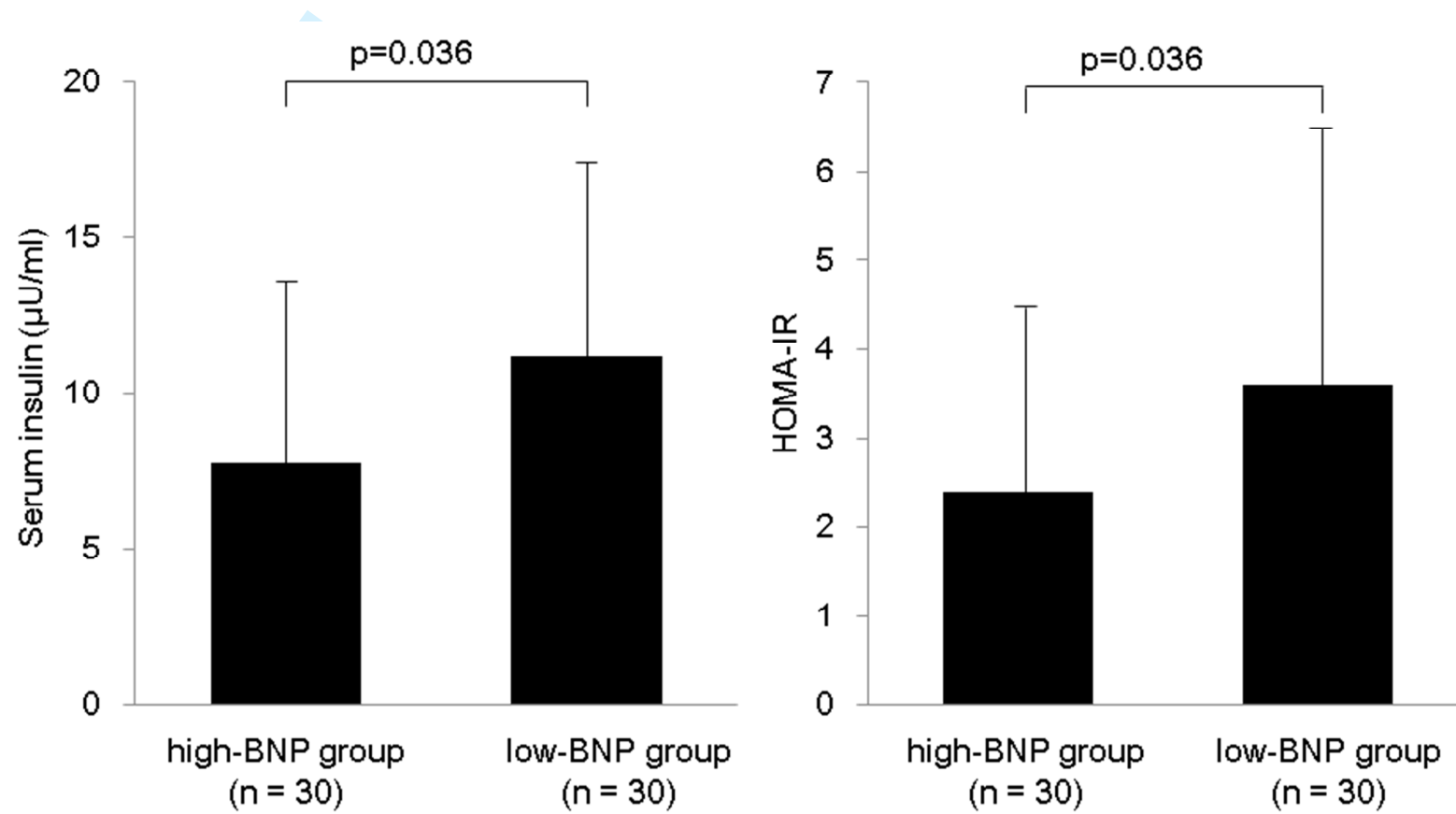


Figure 2

BMJ Open

A significant association of daily physical activity with plasma B-type natriuretic peptide in patients with glucose intolerance: a cross-sectional study

Journal:	<i>BMJ Open</i>
Manuscript ID:	bmjopen-2014-006276.R1
Article Type:	Research
Date Submitted by the Author:	25-Nov-2014
Complete List of Authors:	Hamasaki, Hidetaka; National Center for Global Health and Medicine Kohnodai Hospital, Department of Internal Medicine Yanai, Hidekatsu; National Center for Global Health and Medicine Kohnodai Hospital, Department of Internal Medicine Kakei, Masafumi; Jichi Medical University School of Medicine, Noda, Mitsuhiro; Center Hospital, National Center for Global Health and Medicine, Ezaki, Osamu; Showa Women's University,
Primary Subject Heading:	Diabetes and endocrinology
Secondary Subject Heading:	Diabetes and endocrinology
Keywords:	DIABETES & ENDOCRINOLOGY, SPORTS MEDICINE, PUBLIC HEALTH

SCHOLARONE™
Manuscripts

only

Research**A significant association of daily physical activity with plasma B-type natriuretic peptide in patients with glucose intolerance: a cross-sectional study in National Center for Global Health and Medicine**

Hidetaka Hamasaki,^{1,2} Hidekatsu Yanai,¹ Masafumi Kakei,² Mitsuhiro Noda,³ Osamu Ezaki⁴

¹ Department of Internal Medicine, National Center for Global Health and Medicine Kohnodai Hospital, Chiba, Japan.

² Division of Complementary Medicine, First Department of General Medicine, Saitama Medical Center, Jichi Medical University School of Medicine, Saitama, Japan.

³ Department of Diabetes and Metabolic Medicine, Center Hospital, National Center for Global Health and Medicine, Tokyo, Japan.

⁴ Department of Human Health and Design, Faculty of Human Life and Environmental Sciences, Showa Women's University, Tokyo, Japan.

Correspondence to

Hidekatsu Yanai, MD, PhD, FACP, Department of Internal Medicine, National Center
for Global Health and Medicine Kohnodai Hospital, 1-7-1 Kohnodai, Chiba 272-8516,
Japan. E-mail: dyanai@hospk.ncgm.go.jp; Telephone: +81-47-372-3501; Fax:
+81-47-372-1858

Keywords: B-type natriuretic peptide; glucose intolerance; insulin resistance; physical
activity; type 2 diabetes

Word count: 2,732 words

ABSTRACT

Objectives: In spite of accumulating evidences suggesting an inverse association between insulin resistance and plasma B-type natriuretic peptide (BNP) levels, the effect of daily physical activity on plasma BNP in individuals with glucose intolerance remains unknown. We investigated the association of physical activity level (PAL) with plasma BNP in patients with impaired fasting glucose, impaired glucose tolerance and type 2 diabetes.

Design: Cross-sectional study.

Setting: Outpatients visiting National Center for Global Health and Medicine Kohnodai Hospital.

Participants: A total of 60 patients with glucose intolerance who did not take any hypoglycemic agents, cholesterol lowering agents and antihypertensive agents were recruited. Patients who were diagnosed as having heart failure and renal impairment, engaged in sports-like exercise and resistance training were excluded.

Primary outcome measures: PAL was objectively measured by a triaxial accelerometer. The association between PAL and plasma BNP levels was assessed by multiple regression analysis.

Results: PAL was positively correlated with plasma BNP levels ($r=0.296$, $p=0.021$).

1
2
3
4
5
6 PAL was still significantly correlated with plasma BNP levels after adjustment for age
7
8
9 ($\beta=0.290$, $p=0.014$), and adjustment for age and BMI ($\beta=0.282$, $p=0.018$). Plasma BNP
10
11
12 levels were inversely correlated with serum insulin levels ($r=-0.350$, $p=0.006$) and
13
14 homeostasis model assessment-estimated insulin resistance (HOMA-IR) ($r=-0.363$,
15
16 $p=0.004$). Serum insulin levels (mean \pm SD, 8.1 ± 6.4 $\mu\text{U/ml}$) and HOMA-IR ($2.4 \pm$
17
18 1.9) in high-BNP group were significantly lower than those (11.2 ± 7.4 $\mu\text{U/ml}$ and $3.7 \pm$
19
20 3.0 , respectively) in low-BNP group.
21
22
23
24

25
26 **Conclusions:** Our findings propose the possibility that plasma BNP may be increased
27
28
29 by daily physical activity and BNP is associated with insulin resistance.
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

Strengths and limitations of this study

- This study provides novel data: objectively measured light-intensity daily physical activity using a triaxial accelerometer is positively associated with plasma BNP levels in patients with glucose intolerance.
- Our study evaluated precisely an association between daily physical activity and plasma BNP levels, by recruiting drug-naive patients who were not engaged in sports-like exercise.
- Previous studies reported an inverse association between plasma BNP levels and the markers for insulin resistance in healthy subjects. To our knowledge, our study is the first to show a significant association between plasma BNP levels and insulin resistance in patients with glucose intolerance.
- The limitations are the small sample size and the cross-sectional design which does not allow us to establish any causal relationship.
- Even a triaxial accelerometer may lead to under or overestimation of energy expenditure. It can be denied that energy expenditure assessed by a triaxial accelerometer differ from the true amounts.

INTRODUCTION

B-type natriuretic peptide (BNP) belongs to the cardiac natriuretic peptide family which are released from the heart in response to pressure and volume overload.¹ Plasma BNP and N-terminal proBNP (Nt-proBNP) are increased with the severity of left ventricular dysfunction and/or hypertrophy, therefore, it has become a useful biomarker for diagnosis and prognosis of heart failure with or without type 2 diabetes.²⁻⁷

Plasma BNP levels have been reported to be inversely related to body mass index (BMI) and waist circumference in individuals without heart failure.⁸⁻⁹ Very recently, Olsen MH, et al. reported that Nt-proBNP was significantly lower in individuals with the metabolic syndrome as compared with those without the metabolic syndrome.¹⁰ In their study, Nt-proBNP levels were inversely correlated with BMI, waist circumference, serum cholesterol and triglyceride, plasma glucose and insulin, independently of age and gender.¹⁰ These results suggest that BNP is beneficially associated with obesity and obesity-related metabolic disorders.

Low physical activity and sedentary lifestyle induce the development of type 2 diabetes, and also deteriorate glucose control in patients with type 2 diabetes.¹¹⁻¹² Exercise such as bicycle and hand-grip exercise has been reported to acutely increase plasma BNP levels with exercise intensity, in healthy individuals.¹³⁻¹⁴ However, the

1
2
3
4
5
6 effect of daily physical activity on plasma BNP levels remain obscure. To our
7
8
9 knowledge, there were no previous studies that investigated the association between
10
11
12 daily physical activity and plasma BNP levels in patients with glucose intolerance.
13

14
15 Here, we studied the association of physical activity level (PAL) which was
16
17 evaluated objectively by using a triaxial accelerometer, with plasma BNP levels, in
18
19 patients with impaired fasting glucose (IFG), impaired glucose tolerance (IGT) and type
20
21 2 diabetes. Furthermore, we elucidated the correlations of plasma BNP levels to
22
23 metabolic parameters in patients with such glucose intolerance.
24
25
26
27
28
29
30
31

32 **MATERIAL AND METHODS**

33 34 **Study protocol and participants**

35
36
37 The study protocol was approved by the Medical Ethics Committee of National Center
38
39 for Global Health and Medicine (reference number NCGM-G-001212). We studied 60
40
41 participants (28 men and 32 women) who did not take any hypoglycemic agents,
42
43
44 cholesterol lowering agents and antihypertensive agents. To understand the effects of
45
46
47 daily physical activity such as non-exercise activity thermogenesis (NEAT)¹⁵ on BNP
48
49
50 and metabolic parameters, participants who were engaged in sports-like exercise and
51
52
53 resistance training were excluded. Participants with heart failure and renal impairment
54
55
56
57
58
59
60

1
2
3
4
5
6 were also excluded. The participants were diagnosed as having type 2 diabetes
7
8
9 according to diagnostic criteria set to serum levels of hemoglobin A1c (HbA1c) \geq
10
11
12 6.5 %, fasting plasma glucose (FPG) \geq 126 mg/dl, casual plasma glucose \geq 200
13
14 mg/dl and 2-h values in the 75 g oral glucose tolerance test (OGTT) \geq 200 mg/dl.¹⁶
15
16
17 The participants were defined as having IFG and IGT according to FPG levels 110
18
19 mg/dl to 125 mg/dl, or 2-h values in the OGTT of 140 mg/dl to 199 mg/dl.¹⁶
20
21
22
23
24
25

26 **Anthropometric and physiological measurements**

27
28
29 Height and weight were measured using a rigid stadiometer and scales (DP-7100PW,
30
31 Yamato Co., Ltd, Hyogo, Japan). Waist circumference around the navel was measured
32
33 with a metal anthropometric tape while participants are standing and breathing out. BMI
34
35 was calculated as the body weight in kilograms divided by the height in meters squared.
36
37
38 Blood pressure was measured with participants in a seated position using an automatic
39
40 sphygmomanometer (HEM-762, Omron Co., Ltd, Kyoto, Japan).
41
42
43
44
45
46
47
48

49 **Physical activity measurement**

50
51
52 Daily physical activity was measured using a triaxial accelerometer (Active Style Pro
53
54 HJA-350IT, Omron Co., Ltd, Kyoto, Japan), 74 x 46 x 34 mm and 60 g including
55
56
57
58
59
60

1
2
3
4
5
6 batteries. Participants studied wore the accelerometer on the left side of the waist.
7
8
9 Anteroposterior, mediolateral and vertical acceleration measurements were obtained
10
11 during each physical activity at a rate of 32 Hz to 12-bit accuracy. Each of three signals
12
13 from the triaxial accelerometer was passed through a high-pass filter with a cut-off
14
15 frequency of 0.7 Hz to remove the gravitational acceleration component. The ratios of
16
17 unfiltered to filtered total acceleration (TAU/TAF) and filtered vertical and horizontal
18
19 acceleration (VAF/HAF) were calculated to determine the cut-off value for the
20
21 classification of locomotive activities and non-locomotive activities including such as
22
23 household and occupational activities, which resulted in almost 100% accurate
24
25 demarcation for daily eleven different activities.¹⁷ Furthermore, metabolic equivalent
26
27 values (METs) determined by this triaxial accelerometer have been reported to be
28
29 closely correlated with METs calculated by using energy expenditure (EE) measured by
30
31 indirect calorimetry.^{17 18}
32
33
34
35
36
37
38
39
40
41
42
43

44 Participants studied wore the accelerometer on the left side of the waist for
45
46 consecutive 7 days, and physical activities were recorded. Participants were requested to
47
48 wear the accelerometer except under special circumstances such as sleeping, bathing
49
50 and during aquatic activities. Activity data were stored on a minute-by-minute basis and
51
52 were downloaded to a personal computer before analysis. We excluded days in which
53
54
55
56
57
58
59
60

1
2
3
4
5
6 participants did not wear the accelerometer for more than 8 hours from the data for
7
8
9 analysis.

10
11 Basal metabolic rate (BMR) was estimated from multiple regression equation
12 including age, sex, height and ideal body weight (IBW) as variables, the equation as
13 follows: $BMR \text{ (kcal/day)} = [(0.1283 + 0.0481 \times IBW \text{ (kg)} + 0.0234 \times \text{height (cm)} -$
14
15
16
17
18 $0.0138 \times \text{age (year)} - 0.5473 \times \text{sex coefficient (man: 1, woman:2)}] \times 293$.¹⁹ Total
19
20
21 energy expenditure (TEE) was calculated by manufactured regression equation using
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
METs assessed by the triaxial accelerometer.¹⁸ Physical activity level (PAL) was
calculated by the following equation. $PAL = TEE / BMR$.²⁰

Blood examination, BNP measurements

After a 12-h overnight fast, blood samples were taken from the antecubital vein and
collected into tubes. We measured FPG, HbA1c and serum insulin. FPG was measured
using an enzymatic method (Wako Pure Chemical Industries, Osaka, Japan). Serum
insulin and HbA1c were measured by automated enzyme-linked immunosorbent assays
(TOSOH, Tokyo, Japan). We used homeostasis model assessment-estimated insulin
resistance (HOMA-IR) as the marker for insulin resistance.²¹ Plasma BNP (BNP 1-32)
levels were measured using specific immunoradiometric assay for human BNP

1
2
3
4
5
6 (ARCHITECT BNP-JP[®], ABBOTT JAPAN Co., Ltd, Tokyo, Japan).²² Imprecision
7
8
9 studies yielded within run CVs of 1.1 to 5.1% and total CVs of 2.3 to 5.3% using human
10
11
12 plasma based multi-constituent controls at concentrations of 92, 500, and 3500 ng/L.²²
13

14 15 **Statistical analysis**

16
17 Data were expressed as the mean \pm standard deviation (SD). Correlations among BNP,
18
19 age, BMI, waist circumference, blood pressure, FPG, HbA1c, serum insulin levels,
20
21 HOMA-IR and PAL were assessed by using the Spearman's rank correlation coefficient.
22
23
24 Multiple regression analysis was performed to test the independent correlation of PAL
25
26 with plasma BNP levels. We divided participants into high-BNP group (≥ 8.0 pg/ml)
27
28 and low-BNP group (< 8.0 pg/ml) by the median values of BNP. Differences in serum
29
30 insulin levels, HOMA-IR and PAL between high-BNP group and low-BNP group were
31
32 analyzed by the Mann-Whitney U test. The statistical analyses were performed by using
33
34 SPSS version 19 (IBM Co., Ltd, Chicago, USA). P value < 0.05 was considered to be
35
36 statistically significant.
37
38
39
40
41
42
43
44
45
46
47
48

49 **RESULTS**

50
51 **Table 1** presents the clinical characteristics of 60 participants (28 men and 32 women).
52
53
54
55 31 participants were diagnosed as having type 2 diabetes, 14 participants were with IFG
56
57
58
59
60

and 15 participants were with IGT. The range of age in participants studied was 27-74 years old.

Table 1 Clinical and biochemical characteristics of subjects

n	60
Age (years)	54.7 ± 12.2
Height (cm)	161.3 ± 8.9
Weight (kg)	69.2 ± 16.1
BMI (kg/m ²)	26.5 ± 5.1
Waist circumference (cm)	92.4 ± 13.1
Systolic blood pressure (mmHg)	128.8 ± 17.6
Diastolic blood pressure (mmHg)	80.5 ± 12.5
Fasting plasma glucose (mg/dl)	122.9 ± 25.6
HbA1c (%)	6.7 ± 1.1
BNP (pg/ml)	14.0 ± 14.6
Serum insulin (μU/ml)	9.6 ± 6.9
HOMA-IR	3.02 ± 2.62

Data are expressed as the mean ± SD. BMI, body mass index; BNP, B-type

1
2
3
4
5
6 natriuretic peptide; HOMA-IR, homeostasis model assessment-estimated
7
8
9 insulin resistance.

10
11
12
13
14
15 PAL evaluated by a triaxial accelerometer was significantly and positively
16
17 correlated with plasma BNP levels ($r=0.296$, $p=0.021$; **Figure 1**). Age was correlated
18
19 with BMI ($r=-0.431$, $p<0.001$), but not correlated with PAL ($r=0.096$, $p=0.462$). BMI
20
21 was not correlated with PAL ($r=-0.11$, $p=0.4$). The values of PAL showed a normal
22
23 distribution ($p=0.453$ by Shapiro-Wilk test), however, plasma BNP levels showed
24
25 non-normal distribution ($p<0.001$). Multivariate logistic regression was used after
26
27 controlling simultaneously for potential confounders. Variables considered in the
28
29 models were age (continuous) and BMI (continuous). To adjust the correlation between
30
31 PAL and plasma BNP by age and BMI, plasma BNP values were logarithmically
32
33 transformed. PAL was still significantly correlated with log plasma BNP levels after
34
35 adjustment for age ($\beta=0.290$, $p=0.014$), and adjustment for age and BMI ($\beta=0.282$,
36
37 $p=0.018$).

38
39
40
41
42
43
44
45
46
47
48
49 **Table 2** shows the correlations of plasma BNP levels to clinical and metabolic
50
51 parameters in participants.
52
53
54
55
56
57
58
59
60

Table 2 Correlations of plasma BNP levels to clinical and metabolic parameters

	correlation coefficient	<i>P</i> Value
Age (years)	0.441	<0.001
BMI (kg/m ²)	-0.256	0.048
Waist circumference (cm)	-0.279	0.031
Systolic blood pressure (mmHg)	0.175	0.185
Diastolic blood pressure (mmHg)	-0.070	0.598
Fasting plasma glucose (mg/dl)	-0.237	0.068
HbA1c (%)	0.050	0.705
Serum insulin (μU/ml)	-0.350	0.006
HOMA-IR	-0.363	0.004

BMI, body mass index; BNP, B-type natriuretic peptide; HOMA-IR, homeostasis model assessment-estimated insulin resistance. A statistical analysis was performed by the Spearman's rank correlation coefficient.

There were no significant correlations of plasma BNP levels with systolic and diastolic blood pressure, FPG and HbA1c. Plasma BNP levels were significantly and

1
2
3
4
5
6 inversely correlated with BMI, waist circumference, serum insulin levels and
7
8
9 HOMA-IR, and were positively correlated with age. Significant correlations of plasma
10
11
12 BNP levels with serum insulin ($\beta=-0.204$, $p=0.119$) and HOMA-IR ($\beta=-0.271$,
13
14
15 $p=0.129$) were lost after adjusting for PAL.

16
17
18 To further understand the associations of plasma BNP levels with insulin
19
20
21 resistance, we examined the differences in serum insulin levels and HOMA-IR between
22
23
24 high-BNP group and low-BNP group (**Figure 2**). Serum insulin levels (mean \pm SD, 8.1
25
26 ± 6.4 U/ml) in high-BNP group were significantly lower than those (11.2 ± 7.4 U/ml) in
27
28
29 low-BNP group. HOMA-IR (2.4 ± 1.9) in high-BNP group were also significantly lower
30
31
32 than those (3.7 ± 3.0) in low-BNP group. We also analyzed the relationship between
33
34
35 quartiles of plasma BNP versus serum insulin and HOMA-IR (**Figure 3**). We found a
36
37
38 significant influence of plasma BNP on both serum insulin ($p=0.018$ by the
39
40
41 Kruskal-Wallis ANOVA) and HOMA-IR ($p=0.018$). Serum insulin level in the highest
42
43
44 plasma BNP quartile was significantly lower than in the lowest plasma BNP quartile.
45
46
47 HOMA-IR in the highest plasma BNP quartile was also significantly lower than in the
48
49
50 lowest plasma BNP quartile.

51
52
53 The mean \pm SD of plasma BNP levels in patients with type 2 diabetes (13 men
54
55
56 and 18 women), IFG (5 men and 9 women) and IGT (10 men and 5 women) were 14.3
57
58
59
60

1
2
3
4
5
6 ± 13.5 pg/ml, 12.9 ± 17.2 pg/ml and 14.5 ± 15.1 pg/ml, respectively. The mean \pm SD of
7
8
9 PAL in patients with type 2 diabetes, IFG and IGT were 1.66 ± 0.22 , 1.65 ± 0.15 and
10
11 1.66 ± 0.20 , respectively. There were no significant differences in plasma BNP levels
12
13 and PAL among patients with type 2 diabetes, IFG and IGT.
14
15
16
17
18
19

20 **DISCUSSION**

21
22
23 Recent cross-sectional studies reported that plasma BNP or NT-proBNP levels were
24
25 inversely associated with visceral fat,²³ BMI, waist circumference and serum insulin
26
27 levels.^{9 10} However, the effect of daily physical activity on plasma BNP levels remains
28
29 unknown. Previous cross-sectional studies have not ever been performed by using
30
31 individuals with glucose intolerance as our study participants. In the present study, we
32
33 examined the correlation of daily physical activity measured objectively by a triaxial
34
35 accelerometer with plasma BNP levels, in patients with prediabetes and early untreated
36
37 type 2 diabetes.
38
39
40
41
42
43
44
45

46 Present study demonstrates a significant and positive association between daily
47
48 physical activity and plasma BNP levels, and also an inverse association of plasma BNP
49
50 levels with BMI, waist circumference, serum insulin levels and HOMA-IR. Previous
51
52 studies reported that exercise such as bicycle and hand-grip exercise acutely increase
53
54
55
56
57
58
59
60

1
2
3
4
5
6 plasma BNP levels with exercise intensity in healthy individuals.^{13 14} However, the
7
8
9 effect of daily physical activity on plasma BNP levels remains unknown. It is
10
11
12 noteworthy that we could observe a significant effect of low-intensity daily physical
13
14 activity, including walking, dishing and washing clothes, defined as NEAT,¹⁵ on plasma
15
16 BNP levels. Chainani-Wu N, et al. previously conducted a nested prospective cohort
17
18 study of a lifestyle intervention, to understand the effects of lifestyle on BNP. They
19
20
21 observed the change in BNP for 3 months was significantly associated with the change
22
23 in exercise score, even after controlling the percentage of change in BMI, which agreed
24
25
26 with a significant and positive correlation between PAL and plasma BNP levels after
27
28
29 adjustment by age and BMI in our study. At 3 months in their study, they found an
30
31
32 inverse association of the change in BNP with the changes in BMI and insulin,²⁴
33
34
35 suggesting a significant association between BNP and insulin resistance.
36
37
38

39
40
41 An inverse association between natriuretic peptide and obesity and/or insulin
42
43 resistance has been established.^{8-10 23 25 26} However, controversy exists as to this
44
45
46 association. Lower natriuretic peptide levels in obese individuals has been reported to
47
48
49 be due to increased clearance by the natriuretic peptide receptors (NPR)-C abundantly
50
51
52 expressed in adipose tissue, and also due to decreased natriuretic peptide release from
53
54
55 the heart.²⁷⁻²⁹ BNP binds to NPR on adipose tissue and stimulate lipolysis,³⁰ regulates
56
57
58
59
60

1
2
3
4
5
6 adipose tissue by activation of proliferator-activated receptor gamma (PPAR γ) gene
7
8 expression³¹ and increases adiponectin secretion,³² which improves insulin resistance.

9
10
11 Evidences for the association of glucose intolerance with BNP are very limited. A
12
13 previous study reported that diabetes was significantly associated with low plasma BNP
14
15 levels (adjusted OR 1.51 (1.00 to 2.27) for men; adjusted OR 1.95 (1.26 to 3.02) for
16
17 women).⁸ However, the underlying mechanisms for low BNP levels in patients with
18
19 impaired glucose metabolism remain unknown. In present study, PAL was positively
20
21 correlated with plasma BNP levels, which was inversely correlated with the marker for
22
23 insulin resistance. Furthermore, serum insulin levels and HOMA-IR were significantly
24
25 lower in high-BNP group as compared with those in low-BNP group. BMI and waist
26
27 circumference were also correlated with plasma BNP levels. Increased PAL may be
28
29 associated with reduction of BMI and waist circumference, which improves insulin
30
31 resistance and induce elevation of plasma BNP, due to decreased clearance by NPR-C
32
33 abundantly expressed in adipose tissue. Otherwise, increased plasma BNP may
34
35 ameliorate insulin resistance through various mechanisms, such as reduced oxidative
36
37 stress,³³ decreased systemic inflammation,³⁴ stimulated lipolysis,³⁰ activation of PPAR γ
38
39 gene expression and increased adiponectin secretion.^{31 32} Recently, increased plasma
40
41 BNP levels and/or biological activity could also improve insulin resistance by
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

1
2
3
4
5
6 increasing mitochondrial fat oxidative capacity in white and brown fat, as well as in
7
8 skeletal muscle.^{35 36} However, the cardiac ejection fraction is also inversely related
9
10 with plasma BNP levels but is not beneficial,²⁻⁷ and plasma BNP levels are reported to
11
12 increase lipolysis,³⁷ and to be positively associated insulin resistance.³⁸ The
13
14 relationships among PAL, BNP and insulin resistance in impaired glucose metabolism
15
16 remain largely unknown. We should perform further studies, preferably using a greater
17
18 number of patients with glucose intolerance.
19
20
21
22
23
24

25
26 There are several limitations that need to be considered when interpreting the
27
28 results of this study. This is a cross-sectional study, limiting inferences of causality and
29
30 its direction. Although we controlled for some confounding factors (age, sex, BMI,
31
32 engagement in sports-like exercise or resistance training, and medication), other factors
33
34 such as genetic variation were not taken into account. Furthermore, we studied only
35
36 patients with IFG, IGT and type 2 diabetes, therefore, our conclusions cannot apply to
37
38 healthy populations. A heterogeneous group of individuals with IFG, IGT and type 2
39
40 diabetes was examined. We could not mention that the associations between BNP and
41
42 physical activity or insulin resistance were the same in three groups. We will study these
43
44 associations, by using a great number of patients with each type of glucose intolerance,
45
46 in the future. The triaxial accelerometer is an extensively validated device for evaluating
47
48
49
50
51
52
53
54
55
56
57
58
59
60

1
2
3
4
5
6 physical activity under free living conditions,³⁹⁻⁴¹ however, Leenders et al indicated that
7
8
9 the predictive equations based on the relationship between acceleration and energy
10
11
12 expenditure (EE) during locomotive movements led to under- and overestimation of
13
14
15 TEE.⁴² It is possible that EE and PAL assessed by a triaxial accelerometer differ from
16
17
18 the true amounts of EE and PAL.

19
20
21 In conclusion, we found a significant and positive association between PAL and
22
23
24 plasma BNP levels in patients with prediabetes and early untreated type 2 diabetes.
25
26
27 Plasma BNP levels were inversely associated with insulin resistance. Our findings
28
29
30 propose the possibility that BNP could be increased by daily physical activity and
31
32
33 plasma BNP is beneficially associated with insulin resistance.
34
35
36
37

38 **Contributors**

39
40
41 The study was carried out in collaboration between all authors. HH, HY and OE
42
43
44 conceived and designed the study. HH and MN performed the study. HH, HY and MK
45
46
47 analyzed the data, interpreted the results and wrote the manuscript. HH and HY also
48
49
50 discussed analyses, interpretation, presentation and participated in drafting the
51
52
53 manuscript.
54
55
56
57
58
59
60

Funding

This study was supported by a grant from the National Center for Global Health and Medicine (25-203).

Competing interests

The authors declare that there are no conflicts of interest.

Ethics approval

The study was approved by the Medical Ethics Committee of National Center for Global Health and Medicine (reference number NCGM-G-001212).

Provenance and peer review

Not commissioned; externally peer reviewed.

Data sharing statement

No additional data are available.

REFERENCES

- 1
2
3
4
5
6 1. Gardner DG, Chen S, Glenn DJ, Grigsby CL. Molecular biology of the natriuretic
7
8 peptide system: implications for physiology and hypertension. *Hypertension*
9
10 2007;49:419-26.
11
- 12
13
14 2. Maeda K, Tsutamoto T, Wada A, et al. High levels of plasma brain natriuretic
15
16 peptide and interleukin-6 after optimized treatment for heart failure are independent risk
17
18 factors for morbidity and mortality in patients with congestive heart failure. *J Am Coll*
19
20 *Cardiol* 2000;36:1587-93.
21
22
- 23
24
25 3. Wang TJ, Larson MG, Levy D, et al. Plasma natriuretic peptide levels and the risk of
26
27 cardiovascular events and death. *N Engl J Med* 2004;350:655-63.
28
29
- 30
31
32 4. Alehagen U, Lindstedt G, Levin LA, Dahlström U. Risk of cardiovascular death in
33
34 elderly patients with possible heart failure. B-type natriuretic peptide (BNP) and the
35
36 aminoterminal fragment of ProBNP (N-terminal proBNP) as prognostic indicators in a
37
38 6-year follow-up of a primary care population. *Int J Cardiol* 2005;100:125-33.
39
40
41
- 42
43
44 5. Kroon MH, van den Hurk K, Alsema M, et al. Prospective associations of B-type
45
46 natriuretic peptide with markers of left ventricular function in individuals with and
47
48 without type 2 diabetes: an 8-year follow-up of the Hoorn Study. *Diabetes Care*
49
50 2012;35:2510-14.
51
52
- 53
54
55 6. Olsen MH, Wachtell K, Tuxen C, et al. N-terminal pro-brain natriuretic peptide
56
57
58
59
60

1
2
3
4
5
6 predicts cardiovascular events in patients with hypertension and left ventricular
7
8 hypertrophy: a LIFE study. *J Hypertens* 2004;22:1597-604.

9
10
11 7. Schirmer H, Omland T. Circulating N-terminal pro-atrial natriuretic peptide is an
12 independent predictor of left ventricular hypertrophy in the general population. The
13 Tromsø Study. *Eur Heart J* 1999;20:755-63.

14
15
16 8. Wang TJ, Larson MG, Levy D, et al. Impact of obesity on plasma natriuretic peptide
17 levels. *Circulation* 2004;109:594-600.

18
19
20 9. Koizumi M, Watanabe H, Kaneko Y, et al. Impact of obesity on plasma B-type
21 natriuretic peptide levels in Japanese community-based subjects. *Heart Vessels* 2012;
22 27:287-294.

23
24
25 10. Olsen MH, Hansen TW, Christensen MK, et al. N-terminal pro brain natriuretic
26 peptide is inversely related to metabolic cardiovascular risk factors and the metabolic
27 syndrome. *Hypertension* 2005, 46:660-6.

28
29
30 11. Hamilton MT, Hamilton DG, Zderic TW. Role of low energy expenditure and sitting
31 in obesity, metabolic syndrome, type 2 diabetes, and cardiovascular disease. *Diabetes*
32 2007;56:2655-67.

33
34
35 12. Avery L, Flynn D, van Wersch A, Sniehotta FF, Trenell MI. Changing physical
36 activity behavior in type 2 diabetes: a systematic review and meta-analysis of behavioral
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

1
2
3
4
5
6 interventions. *Diabetes Care* 2012;35:2681-89.

7
8
9 13. Barletta G, Stefani L, Del Bene R, et al. Effects of exercise on natriuretic peptides
10
11 and cardiac function in man. *Int J Cardiol* 1998;65:217-25.

12
13
14 14. Nielsen HB, De Palo EF, Meneghetti M, Madsen PL, Ihlemann N, Secher NH.
15
16 Circulating immunoreactive proANP1-30 and proANP31-67 responses to acute exercise.
17
18
19
20
21
22 *Regul Pept* 2001;99:203-7.

23
24 15. Levine JA. Non-exercise activity thermogenesis (NEAT). *Nutr Rev* 2004;62:S82-97.

25
26 16. American Diabetes Association. Diagnosis and classification of diabetes mellitus.
27
28
29
30
31 *Diabetes Care* 2010;33:S62-9.

32
33 17. Oshima Y, Kawaguchi K, Tanaka S, et al. Classifying household and locomotive
34
35
36
37 activities using a triaxial accelerometer. *Gait Posture* 2010;31:370-4.

38
39 18. Ohkawara K, Oshima Y, Hikiyama Y, Ishikawa-Takata K, Tabata I, Tanaka S.
40
41
42
43
44
45
46 Real-time estimation of daily physical activity intensity by a triaxial accelerometer and
47
48
49
50
51 a gravity-removal classification algorithm. *Br J Nutr* 2011;105:1681-91.

52
53 19. Ganpule AA, Tanaka S, Ishikawa-Takata K, Tabata I. Interindividual variability in
54
55
56
57
58
59
60 sleeping metabolic rate in Japanese subjects. *Eur J Clin Nutr* 2007;61:1256-61.

60
20. Energy and protein requirements. Report of a joint FAO/WHO/UNU Expert
Consultation. *World Health Organ Tech Rep Ser* 1985;724:1-206.

- 1
2
3
4
5
6 21. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC.
7
8 Homeostasis model assessment: insulin resistance and beta-cell function from fasting
9
10 plasma glucose and insulin concentrations in man. *Diabetologia* 1985;28:412-419.
11
12
13
14 22. Mongia SK, La'ulu SL, Apple FS, Ler R, Murakami MM, Roberts WL. Performance
15
16 characteristics of the Architect brain natriuretic peptide (BNP) assay: a two site study.
17
18
19
20
21 *Clin Chim Acta* 2008;391:102-5.
22
23
24 23. Neeland IJ, Winders BR, Ayers CR, et al. Higher natriuretic peptide levels associate
25
26 with a favorable adipose tissue distribution profile. *J Am Coll Cardiol* 2013;62:752-60.
27
28
29 24. Chainani-Wu N, Weidner G, Purnell DM, et al. Relation of B-type natriuretic
30
31 peptide levels to body mass index after comprehensive lifestyle changes. *Am J Cardiol*
32
33
34
35 2010;105:1570-6.
36
37
38 25. Mehra MR, Uber PA, Park MH, et al. Obesity and suppressed B-type natriuretic
39
40 peptide levels in heart failure. *J Am Coll Cardiol* 2004;43:1590-5.
41
42
43 26. McCord J, Mundy BJ, Hudson MP, et al. Relationship between obesity and B-type
44
45 natriuretic peptide levels. *Arch Intern Med* 2004;164:2247-52.
46
47
48
49 27. Dessì-Fulgheri P, Sarzani R, Tamburrini P, et al. Plasma atrial natriuretic peptide and
50
51 natriuretic peptide receptor gene expression in adipose tissue of normotensive and
52
53
54
55 hypertensive obese patients. *J Hypertens* 1997;15:1695-9.
56
57
58
59
60

- 1
2
3
4
5
6 28. Das SR, Drazner MH, Dries DL, et al. Impact of body mass and body composition
7
8 on circulating levels of natriuretic peptides: results from the Dallas Heart Study.
9
10 *Circulation* 2005;112:2163-8.
11
12
13
14 29. Costello-Boerrigter LC, Burnett JC Jr. A new role for the natriuretic peptides:
15
16 metabolic regulators of the adipocyte. *J Am Coll Cardiol* 2009;53:2078-9.
17
18
19
20 30. Sengenès C, Berlan M, De Glisezinski I, Lafontan M, Galitzky J. Natriuretic
21
22 peptides: a new lipolytic pathway in human adipocytes. *FASEB J* 2000;14:1345-51.
23
24
25
26 31. Miyashita K, Itoh H, Tsujimoto H, et al. Natriuretic
27
28 peptides/cGMP/cGMP-dependent protein kinase cascades promote muscle
29
30 mitochondrial biogenesis and prevent obesity. *Diabetes* 2009;58:2880-92.
31
32
33
34 32. Tsukamoto O, Fujita M, Kato M, et al. Natriuretic peptides enhance the production
35
36 of adiponectin in human adipocytes and in patients with chronic heart failure. *J Am Coll*
37
38 *Cardiol* 2009;53:2070-7.
39
40
41
42
43 33. Rajagopalan S, Kurz S, Münzel T, et al. Angiotensin II-mediated hypertension in the
44
45 rat increases vascular superoxide production via membrane NADH/NADPH oxidase
46
47 activation. Contribution to alterations of vasomotor tone. *J Clin Invest*
48
49
50
51 1996;97:1916-23.
52
53
54
55 34. Moro C, Klimcakova E, Lolmède K, et al. Atrial natriuretic peptide inhibits the
56
57
58
59
60

1
2
3
4
5
6 production of adipokines and cytokines linked to inflammation and insulin resistance in
7
8 human subcutaneous adipose tissue. *Diabetologia* 2007;50:1038-47.

9
10
11 35. Bordicchia M, Liu D, Amri EZ, et al. Cardiac natriuretic peptides act via p38 MAPK
12
13 to induce the brown fat thermogenic program in mouse and human adipocytes. *J Clin*
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

Invest 2012;122:1022-36.

36. Engeli S, Birkenfeld AL, Badin PM, et al. Natriuretic peptides enhance the oxidative
capacity of human skeletal muscle. *J Clin Invest* 2012;122:4675-9.

37. Polak J, Kotrc M, Wedellova Z, et al. Lipolytic effects of B-type natriuretic peptide
1-32 in adipose tissue of heart failure patients compared with healthy controls. *J Am*
Coll Cardiol 2011;58:1119-25.

38. Tekes S, Cikim AS. The association of brain natriuretic peptide and insulin
resistance in obesity-related hypertension. *J Hum Hypertens* 2007;21:546-50.

39. Plasqui G, Bonomi AG, Westerterp KR. Daily physical activity assessment with
accelerometers: new insights and validation studies. *Obes Rev* 2013;14:451-62.

40. Midorikawa T, Tanaka S, Kaneko K, et al. Evaluation of low-intensity physical
activity by triaxial accelerometry. *Obesity* 2007;15:3031-8.

41. Ekelund U, Sjöström M, Yngve A, et al. Physical activity assessed by activity
monitor and doubly labeled water in children. *Med Sci Sports Exerc* 2001;33:275-81.

1
2
3
4
5
6 42. Leenders NY, Sherman WM, Nagaraja HN. Energy expenditure estimated by
7
8
9 accelerometry and doubly labeled water: do they agree? *Med Sci Sports Exerc*
10
11
12 2006;38:2165-72.
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

For peer review only

(figure legends)

Figure 1 Correlation of physical activity level and plasma BNP levels in 60 subjects.

r indicates correlation coefficient analyzed by the Spearman's rank correlation coefficient.

Figure 2 Serum insulin levels (A) and homeostatic model assessment-estimated insulin resistance (HOMA-IR) (B) in high-BNP group (n = 30) and low-BNP group (n = 30).

Data are mean values±SD. We divided subjects into high-BNP group (≥ 8.0 pg/ml) and low-BNP group (< 8.0 pg/ml) by the median values of BNP. Differences in serum insulin levels and HOMA-IR between high-BNP group and low-BNP group were analyzed by the Mann-Whitney U test.

Figure 3 Serum insulin levels (A) and homeostatic model assessment-estimated insulin resistance (HOMA-IR) (B) in each plasma BNP quartiles. Q1 is the lowest quartile and

Q4 is the highest quartile. Boxes indicate medians and quartiles; whiskers indicate the lowest and highest values. We found a significant influence of plasma BNP on both serum insulin (p=0.018 by the Kruskal-Wallis ANOVA) and HOMA-IR (p=0.018). The difference between Q1 and Q2-4 was statistically analyzed by the Scheffe's F Test.

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

For peer review only

Research

A significant association of daily physical activity with plasma B-type natriuretic peptide in patients with glucose intolerance: a cross-sectional study in National Center for Global Health and Medicine

Hidetaka Hamasaki,^{1,2} Hidekatsu Yanai,¹ Masafumi Kakei,² Mitsuhiro Noda,³ Osamu Ezaki⁴

¹ Department of Internal Medicine, National Center for Global Health and Medicine Kohnodai Hospital, Chiba, Japan.

² Division of Complementary Medicine, First Department of General Medicine, Saitama Medical Center, Jichi Medical University School of Medicine, Saitama, Japan.

³ Department of Diabetes and Metabolic Medicine, Center Hospital, National Center for Global Health and Medicine, Tokyo, Japan.

⁴ Department of Human Health and Design, Faculty of Human Life and Environmental Sciences, Showa Women's University, Tokyo, Japan.

Correspondence to

Hidekatsu Yanai, MD, PhD, FACP, Department of Internal Medicine, National Center
for Global Health and Medicine Kohnodai Hospital, 1-7-1 Kohnodai, Chiba 272-8516,
Japan. E-mail: dyanai@hospk.ncgm.go.jp; Telephone: +81-47-372-3501; Fax:
+81-47-372-1858

Keywords: B-type natriuretic peptide; glucose intolerance; insulin resistance; physical
activity; type 2 diabetes

Word count: 2,732 words

ABSTRACT

Objectives: In spite of accumulating evidences suggesting an inverse association between insulin resistance and plasma B-type natriuretic peptide (BNP) levels, the effect of daily physical activity on plasma BNP in individuals with glucose intolerance remains unknown. We investigated the association of physical activity level (PAL) with plasma BNP in patients with impaired fasting glucose, impaired glucose tolerance and type 2 diabetes.

Design: Cross-sectional study.

Setting: Outpatients visiting National Center for Global Health and Medicine Kohnodai Hospital.

Participants: A total of 60 patients with glucose intolerance who did not take any hypoglycemic agents, cholesterol lowering agents and antihypertensive agents were recruited. Patients who were diagnosed as having heart failure and renal impairment, engaged in sports-like exercise and resistance training were excluded.

Primary outcome measures: PAL was objectively measured by a triaxial accelerometer. The association between PAL and plasma BNP levels was assessed by multiple regression analysis.

Results: PAL was positively correlated with plasma BNP levels ($r=0.296$, $p=0.021$).

1
2
3
4
5
6 PAL was still significantly correlated with plasma BNP levels after adjustment for age
7
8
9 ($\beta=0.290$, $p=0.014$), and adjustment for age and BMI ($\beta=0.282$, $p=0.018$). Plasma BNP
10
11
12 levels were inversely correlated with serum insulin levels ($r=-0.350$, $p=0.006$) and
13
14 homeostasis model assessment-estimated insulin resistance (HOMA-IR) ($r=-0.363$,
15
16 $p=0.004$). Serum insulin levels (mean \pm SD, 8.1 ± 6.4 $\mu\text{U/ml}$) and HOMA-IR ($2.4 \pm$
17
18 1.9) in high-BNP group were significantly lower than those (11.2 ± 7.4 $\mu\text{U/ml}$ and $3.7 \pm$
19
20 3.0 , respectively) in low-BNP group.
21
22
23
24

25
26 **Conclusions:** Our findings propose the possibility that plasma BNP may be increased
27
28
29 by daily physical activity and BNP is associated with insulin resistance.
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

Strengths and limitations of this study

- This study provides novel data: objectively measured light-intensity daily physical activity using a triaxial accelerometer is positively associated with plasma BNP levels in patients with glucose intolerance.
- Our study evaluated precisely an association between daily physical activity and plasma BNP levels, by recruiting drug-naive patients who were not engaged in sports-like exercise.
- Previous studies reported an inverse association between plasma BNP levels and the markers for insulin resistance in healthy subjects. To our knowledge, our study is the first to show a significant association between plasma BNP levels and insulin resistance in patients with glucose intolerance.
- The limitations are the small sample size and the cross-sectional design which does not allow us to establish any causal relationship.
- Even a triaxial accelerometer may lead to under or overestimation of energy expenditure. It can be denied that energy expenditure assessed by a triaxial accelerometer differ from the true amounts.

INTRODUCTION

B-type natriuretic peptide (BNP) belongs to the cardiac natriuretic peptide family which are released from the heart in response to pressure and volume overload.¹ Plasma BNP and N-terminal proBNP (Nt-proBNP) are increased with the severity of left ventricular dysfunction and/or hypertrophy, therefore, it has become a useful biomarker for diagnosis and prognosis of heart failure with or without type 2 diabetes.²⁻⁷

Plasma BNP levels have been reported to be inversely related to body mass index (BMI) and waist circumference in individuals without heart failure.⁸⁻⁹ Very recently, Olsen MH, et al. reported that Nt-proBNP was significantly lower in individuals with the metabolic syndrome as compared with those without the metabolic syndrome.¹⁰ In their study, Nt-proBNP levels were inversely correlated with BMI, waist circumference, serum cholesterol and triglyceride, plasma glucose and insulin, independently of age and gender.¹⁰ These results suggest that BNP is beneficially associated with obesity and obesity-related metabolic disorders.

Low physical activity and sedentary lifestyle induce the development of type 2 diabetes, and also deteriorate glucose control in patients with type 2 diabetes.¹¹⁻¹² Exercise such as bicycle and hand-grip exercise has been reported to acutely increase plasma BNP levels with exercise intensity, in healthy individuals.¹³⁻¹⁴ However, the

1
2
3
4
5
6 effect of daily physical activity on plasma BNP levels remain obscure. To our
7
8
9 knowledge, there were no previous studies that investigated the association between
10
11
12 daily physical activity and plasma BNP levels in patients with glucose intolerance.
13

14
15 Here, we studied the association of physical activity level (PAL) which was
16
17 evaluated objectively by using a triaxial accelerometer, with plasma BNP levels, in
18
19 patients with impaired fasting glucose (IFG), impaired glucose tolerance (IGT) and type
20
21 2 diabetes. Furthermore, we elucidated the correlations of plasma BNP levels to
22
23 metabolic parameters in patients with such glucose intolerance.
24
25
26
27
28
29
30
31

32 **MATERIAL AND METHODS**

33 34 **Study protocol and participants**

35
36
37 The study protocol was approved by the Medical Ethics Committee of National Center
38
39 for Global Health and Medicine (reference number NCGM-G-001212). We studied 60
40
41 participants (28 men and 32 women) who did not take any hypoglycemic agents,
42
43 cholesterol lowering agents and antihypertensive agents. To understand the effects of
44
45 daily physical activity such as non-exercise activity thermogenesis (NEAT)¹⁵ on BNP
46
47 and metabolic parameters, participants who were engaged in sports-like exercise and
48
49 resistance training were excluded. Participants with heart failure and renal impairment
50
51
52
53
54
55
56
57
58
59
60

1
2
3
4
5
6 were also excluded. The participants were diagnosed as having type 2 diabetes
7
8
9 according to diagnostic criteria set to serum levels of hemoglobin A1c (HbA1c) \geq
10
11
12 6.5 %, fasting plasma glucose (FPG) \geq 126 mg/dl, casual plasma glucose \geq 200
13
14 mg/dl and 2-h values in the 75 g oral glucose tolerance test (OGTT) \geq 200 mg/dl.¹⁶
15
16
17 The participants were defined as having IFG and IGT according to FPG levels 110
18
19 mg/dl to 125 mg/dl, or 2-h values in the OGTT of 140 mg/dl to 199 mg/dl.¹⁶
20
21
22
23
24
25

26 **Anthropometric and physiological measurements**

27
28
29 Height and weight were measured using a rigid stadiometer and scales (DP-7100PW,
30
31 Yamato Co., Ltd, Hyogo, Japan). Waist circumference around the navel was measured
32
33 with a metal anthropometric tape while participants are standing and breathing out. BMI
34
35 was calculated as the body weight in kilograms divided by the height in meters squared.
36
37
38 Blood pressure was measured with participants in a seated position using an automatic
39
40 sphygmomanometer (HEM-762, Omron Co., Ltd, Kyoto, Japan).
41
42
43
44
45
46
47
48

49 **Physical activity measurement**

50
51
52 Daily physical activity was measured using a triaxial accelerometer (Active Style Pro
53
54 HJA-350IT, Omron Co., Ltd, Kyoto, Japan), 74 x 46 x 34 mm and 60 g including
55
56
57
58
59
60

1
2
3
4
5
6 batteries. Participants studied wore the accelerometer on the left side of the waist.
7
8
9 Anteroposterior, mediolateral and vertical acceleration measurements were obtained
10
11 during each physical activity at a rate of 32 Hz to 12-bit accuracy. Each of three signals
12
13 from the triaxial accelerometer was passed through a high-pass filter with a cut-off
14
15 frequency of 0.7 Hz to remove the gravitational acceleration component. The ratios of
16
17 unfiltered to filtered total acceleration (TAU/TAF) and filtered vertical and horizontal
18
19 acceleration (VAF/HAF) were calculated to determine the cut-off value for the
20
21 classification of locomotive activities and non-locomotive activities including such as
22
23 household and occupational activities, which resulted in almost 100% accurate
24
25 demarcation for daily eleven different activities.¹⁷ Furthermore, metabolic equivalent
26
27 values (METs) determined by this triaxial accelerometer have been reported to be
28
29 closely correlated with METs calculated by using energy expenditure (EE) measured by
30
31 indirect calorimetry.^{17 18}
32
33
34
35
36
37
38
39
40
41
42
43

44 Participants studied wore the accelerometer on the left side of the waist for
45
46 consecutive 7 days, and physical activities were recorded. Participants were requested to
47
48 wear the accelerometer except under special circumstances such as sleeping, bathing
49
50 and during aquatic activities. Activity data were stored on a minute-by-minute basis and
51
52 were downloaded to a personal computer before analysis. We excluded days in which
53
54
55
56
57
58
59
60

1
2
3
4
5
6 participants did not wear the accelerometer for more than 8 hours from the data for
7
8
9 analysis.

10
11 Basal metabolic rate (BMR) was estimated from multiple regression equation
12 including age, sex, height and ideal body weight (IBW) as variables, the equation as
13 follows: $BMR \text{ (kcal/day)} = [(0.1283 + 0.0481 \times IBW \text{ (kg)} + 0.0234 \times \text{height (cm)} -$
14
15
16
17
18
19
20
21 $0.0138 \times \text{age (year)} - 0.5473 \times \text{sex coefficient (man: 1, woman:2)}] \times 293$.¹⁹ Total
22
23 energy expenditure (TEE) was calculated by manufactured regression equation using
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
METs assessed by the triaxial accelerometer.¹⁸ Physical activity level (PAL) was
calculated by the following equation. $PAL = TEE / BMR$.²⁰

Blood examination, BNP measurements

After a 12-h overnight fast, blood samples were taken from the antecubital vein and
collected into tubes. We measured FPG, HbA1c and serum insulin. FPG was measured
using an enzymatic method (Wako Pure Chemical Industries, Osaka, Japan). Serum
insulin and HbA1c were measured by automated enzyme-linked immunosorbent assays
(TOSOH, Tokyo, Japan). We used homeostasis model assessment-estimated insulin
resistance (HOMA-IR) as the marker for insulin resistance.²¹ Plasma BNP (BNP 1-32)
levels were measured using specific immunoradiometric assay for human BNP

1
2
3
4
5
6 (ARCHITECT BNP-JP[®], ABBOTT JAPAN Co., Ltd, Tokyo, Japan).²² Imprecision
7
8
9 studies yielded within run CVs of 1.1 to 5.1% and total CVs of 2.3 to 5.3% using human
10
11 plasma based multi-constituent controls at concentrations of 92, 500, and 3500 ng/L.²²

14 15 **Statistical analysis**

16
17 Data were expressed as the mean \pm standard deviation (SD). Correlations among BNP,
18
19 age, BMI, waist circumference, blood pressure, FPG, HbA1c, serum insulin levels,
20
21 HOMA-IR and PAL were assessed by using the Spearman's rank correlation coefficient.
22
23
24 Multiple regression analysis was performed to test the independent correlation of PAL
25
26 with plasma BNP levels. We divided participants into high-BNP group (≥ 8.0 pg/ml)
27
28 and low-BNP group (< 8.0 pg/ml) by the median values of BNP. Differences in serum
29
30 insulin levels, HOMA-IR and PAL between high-BNP group and low-BNP group were
31
32 analyzed by the Mann-Whitney U test. The statistical analyses were performed by using
33
34 SPSS version 19 (IBM Co., Ltd, Chicago, USA). P value < 0.05 was considered to be
35
36 statistically significant.
37
38
39
40
41
42
43
44
45
46
47
48

49 **RESULTS**

50
51 **Table 1** presents the clinical characteristics of 60 participants (28 men and 32 women).
52
53
54
55 31 participants were diagnosed as having type 2 diabetes, 14 participants were with IFG
56
57
58
59
60

and 15 participants were with IGT. The range of age in participants studied was 27-74 years old.

Table 1 Clinical and biochemical characteristics of subjects

n	60
Age (years)	54.7 ± 12.2
Height (cm)	161.3 ± 8.9
Weight (kg)	69.2 ± 16.1
BMI (kg/m ²)	26.5 ± 5.1
Waist circumference (cm)	92.4 ± 13.1
Systolic blood pressure (mmHg)	128.8 ± 17.6
Diastolic blood pressure (mmHg)	80.5 ± 12.5
Fasting plasma glucose (mg/dl)	122.9 ± 25.6
HbA1c (%)	6.7 ± 1.1
BNP (pg/ml)	14.0 ± 14.6
Serum insulin (μU/ml)	9.6 ± 6.9
HOMA-IR	3.02 ± 2.62

Data are expressed as the mean ± SD. BMI, body mass index; BNP, B-type

1
2
3
4
5
6 natriuretic peptide; HOMA-IR, homeostasis model assessment-estimated
7
8
9 insulin resistance.

10
11
12
13
14
15 PAL evaluated by a triaxial accelerometer was significantly and positively
16
17 correlated with plasma BNP levels ($r=0.296$, $p=0.021$; **Figure 1**). Age was correlated
18 with BMI ($r=-0.431$, $p<0.001$), but not correlated with PAL ($r=0.096$, $p=0.462$). BMI
19 was not correlated with PAL ($r=-0.11$, $p=0.4$). The values of PAL showed a normal
20 distribution ($p=0.453$ by Shapiro-Wilk test), however, plasma BNP levels showed
21 non-normal distribution ($p<0.001$). Multivariate logistic regression was used after
22 controlling simultaneously for potential confounders. Variables considered in the
23 models were age (continuous) and BMI (continuous). To adjust the correlation between
24 PAL and plasma BNP by age and BMI, plasma BNP values were logarithmically
25 transformed. PAL was still significantly correlated with log plasma BNP levels after
26
27 adjustment for age ($\beta=0.290$, $p=0.014$), and adjustment for age and BMI ($\beta=0.282$,
28 $p=0.018$).

29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49 **Table 2** shows the correlations of plasma BNP levels to clinical and metabolic
50 parameters in participants.
51
52
53
54
55
56
57
58
59
60

Table 2 Correlations of plasma BNP levels to clinical and metabolic parameters

	correlation coefficient	<i>P</i> Value
Age (years)	0.441	<0.001
BMI (kg/m ²)	-0.256	0.048
Waist circumference (cm)	-0.279	0.031
Systolic blood pressure (mmHg)	0.175	0.185
Diastolic blood pressure (mmHg)	-0.070	0.598
Fasting plasma glucose (mg/dl)	-0.237	0.068
HbA1c (%)	0.050	0.705
Serum insulin (μU/ml)	-0.350	0.006
HOMA-IR	-0.363	0.004

BMI, body mass index; BNP, B-type natriuretic peptide; HOMA-IR, homeostasis model assessment-estimated insulin resistance. A statistical analysis was performed by the Spearman's rank correlation coefficient.

There were no significant correlations of plasma BNP levels with systolic and diastolic blood pressure, FPG and HbA1c. Plasma BNP levels were significantly and

1
2
3
4
5
6 inversely correlated with BMI, waist circumference, serum insulin levels and
7
8
9 HOMA-IR, and were positively correlated with age. Significant correlations of plasma
10
11
12 BNP levels with serum insulin ($\beta=-0.204$, $p=0.119$) and HOMA-IR ($\beta=-0.271$,
13
14 $p=0.129$) were lost after adjusting for PAL.

15
16
17
18 To further understand the associations of plasma BNP levels with insulin
19
20 resistance, we examined the differences in serum insulin levels and HOMA-IR between
21
22 high-BNP group and low-BNP group (**Figure 2**). Serum insulin levels (mean \pm SD, 8.1
23
24 \pm 6.4 U/ml) in high-BNP group were significantly lower than those (11.2 \pm 7.4 U/ml) in
25
26 low-BNP group. HOMA-IR (2.4 \pm 1.9) in high-BNP group were also significantly lower
27
28 than those (3.7 \pm 3.0) in low-BNP group. We also analyzed the relationship between
29
30 quartiles of plasma BNP versus serum insulin and HOMA-IR (Figure 3). We found a
31
32 significant influence of plasma BNP on both serum insulin ($p=0.018$ by the
33
34 Kruskal-Wallis ANOVA) and HOMA-IR ($p=0.018$). Serum insulin level in the highest
35
36 plasma BNP quartile was significantly lower than in the lowest plasma BNP quartile.
37
38 HOMA-IR in the highest plasma BNP quartile was also significantly lower than in the
39
40 lowest plasma BNP quartile.

41
42
43
44
45
46
47
48
49
50
51
52
53 The mean \pm SD of plasma BNP levels in patients with type 2 diabetes (13 men
54
55 and 18 women), IFG (5 men and 9 women) and IGT (10 men and 5 women) were 14.3
56
57
58
59
60

1
2
3
4
5
6 ± 13.5 pg/ml, 12.9 ± 17.2 pg/ml and 14.5 ± 15.1 pg/ml, respectively. The mean ± SD of
7
8
9 PAL in patients with type 2 diabetes, IFG and IGT were 1.66 ± 0.22, 1.65 ± 0.15 and
10
11 1.66 ± 0.20, respectively. There were no significant differences in plasma BNP levels
12
13 and PAL among patients with type 2 diabetes, IFG and IGT.
14
15
16
17
18
19

20 DISCUSSION

21
22
23 Recent cross-sectional studies reported that plasma BNP or NT-proBNP levels were
24
25 inversely associated with visceral fat,²³ BMI, waist circumference and serum insulin
26
27 levels.^{9 10} However, the effect of daily physical activity on plasma BNP levels remains
28
29 unknown. Previous cross-sectional studies have not ever been performed by using
30
31 individuals with glucose intolerance as our study participants. In the present study, we
32
33 examined the correlation of daily physical activity measured objectively by a triaxial
34
35 accelerometer with plasma BNP levels, in patients with prediabetes and early untreated
36
37 type 2 diabetes.
38
39
40
41
42
43
44

45
46 Present study demonstrates a significant and positive association between daily
47
48 physical activity and plasma BNP levels, and also an inverse association of plasma BNP
49
50 levels with BMI, waist circumference, serum insulin levels and HOMA-IR. Previous
51
52 studies reported that exercise such as bicycle and hand-grip exercise acutely increase
53
54
55
56
57
58
59
60

1
2
3
4
5
6 plasma BNP levels with exercise intensity in healthy individuals.^{13 14} However, the
7
8
9 effect of daily physical activity on plasma BNP levels remains unknown. It is
10
11
12 noteworthy that we could observe a significant effect of low-intensity daily physical
13
14 activity, including walking, dishwashing and washing clothes, defined as NEAT,¹⁵ on plasma
15
16 BNP levels. Chainani-Wu N, et al. previously conducted a nested prospective cohort
17
18 study of a lifestyle intervention, to understand the effects of lifestyle on BNP. They
19
20
21 observed the change in BNP for 3 months was significantly associated with the change
22
23 in exercise score, even after controlling the percentage of change in BMI, which agreed
24
25
26 with a significant and positive correlation between PAL and plasma BNP levels after
27
28
29 adjustment by age and BMI in our study. At 3 months in their study, they found an
30
31
32 inverse association of the change in BNP with the changes in BMI and insulin,²⁴
33
34
35 suggesting a significant association between BNP and insulin resistance.
36
37
38

39
40
41 An inverse association between natriuretic peptide and obesity and/or insulin
42
43 resistance has been established.^{8-10 23 25 26} However, controversy exists as to this
44
45
46 association. Lower natriuretic peptide levels in obese individuals has been reported to
47
48
49 be due to increased clearance by the natriuretic peptide receptors (NPR)-C abundantly
50
51
52 expressed in adipose tissue, and also due to decreased natriuretic peptide release from
53
54
55 the heart.²⁷⁻²⁹ BNP binds to NPR on adipose tissue and stimulate lipolysis,³⁰ regulates
56
57
58
59
60

1
2
3
4
5
6 adipose tissue by activation of proliferator-activated receptor gamma (PPAR γ) gene
7
8 expression³¹ and increases adiponectin secretion,³² which improves insulin resistance.

9
10
11 Evidences for the association of glucose intolerance with BNP are very limited. A
12
13 previous study reported that diabetes was significantly associated with low plasma BNP
14
15 levels (adjusted OR 1.51 (1.00 to 2.27) for men; adjusted OR 1.95 (1.26 to 3.02) for
16
17 women).⁸ However, the underlying mechanisms for low BNP levels in patients with
18
19 impaired glucose metabolism remain unknown. In present study, PAL was positively
20
21 correlated with plasma BNP levels, which was inversely correlated with the marker for
22
23 insulin resistance. Furthermore, serum insulin levels and HOMA-IR were significantly
24
25 lower in high-BNP group as compared with those in low-BNP group. BMI and waist
26
27 circumference were also correlated with plasma BNP levels. Increased PAL may be
28
29 associated with reduction of BMI and waist circumference, which improves insulin
30
31 resistance and induce elevation of plasma BNP, due to decreased clearance by NPR-C
32
33 abundantly expressed in adipose tissue. Otherwise, increased plasma BNP may
34
35 ameliorate insulin resistance through various mechanisms, such as reduced oxidative
36
37 stress,³³ decreased systemic inflammation,³⁴ stimulated lipolysis,³⁰ activation of PPAR γ
38
39 gene expression and increased adiponectin secretion.^{31 32} Recently, increased plasma
40
41 BNP levels and/or biological activity could also improve insulin resistance by
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

1
2
3
4
5
6 increasing mitochondrial fat oxidative capacity in white and brown fat, as well as in
7
8 skeletal muscle.^{35 36} However, the cardiac ejection fraction is also inversely related
9
10 with plasma BNP levels but is not beneficial,²⁻⁷ and plasma BNP levels are reported to
11
12 increase lipolysis,³⁷ and to be positively associated insulin resistance.³⁸ The
13
14
15
16
17
18 relationships among PAL, BNP and insulin resistance in impaired glucose metabolism
19
20 remain largely unknown. We should perform further studies, preferably using a greater
21
22
23
24 number of patients with glucose intolerance.

25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

There are several limitations that need to be considered when interpreting the results of this study. This is a cross-sectional study, limiting inferences of causality and its direction. Although we controlled for some confounding factors (age, sex, BMI, engagement in sports-like exercise or resistance training, and medication), other factors such as genetic variation were not taken into account. Furthermore, we studied only patients with IFG, IGT and type 2 diabetes, therefore, our conclusions cannot apply to healthy populations. A heterogeneous group of individuals with IFG, IGT and type 2 diabetes was examined. We could not mention that the associations between BNP and physical activity or insulin resistance were the same in three groups. We will study these associations, by using a great number of patients with each type of glucose intolerance, in the future. The triaxial accelerometer is an extensively validated device for evaluating

1
2
3
4
5
6 physical activity under free living conditions,³⁹⁻⁴¹ however, Leenders et al indicated that
7
8
9 the predictive equations based on the relationship between acceleration and energy
10
11
12 expenditure (EE) during locomotive movements led to under- and overestimation of
13
14
15 TEE.⁴² It is possible that EE and PAL assessed by a triaxial accelerometer differ from
16
17
18 the true amounts of EE and PAL.

19
20
21 In conclusion, we found a significant and positive association between PAL and
22
23
24 plasma BNP levels in patients with prediabetes and early untreated type 2 diabetes.
25
26
27 Plasma BNP levels were inversely associated with insulin resistance. Our findings
28
29
30 propose the possibility that BNP could be increased by daily physical activity and
31
32
33 plasma BNP is beneficially associated with insulin resistance.
34
35
36
37

38 **Contributors**

39
40
41 The study was carried out in collaboration between all authors. HH, HY and OE
42
43
44 conceived and designed the study. HH and MN performed the study. HH, HY and MK
45
46
47 analyzed the data, interpreted the results and wrote the manuscript. HH and HY also
48
49
50 discussed analyses, interpretation, presentation and participated in drafting the
51
52
53 manuscript.
54
55
56
57
58
59
60

Funding

This study was supported by a grant from the National Center for Global Health and Medicine (25-203).

Competing interests

The authors declare that there are no conflicts of interest.

Ethics approval

The study was approved by the Medical Ethics Committee of National Center for Global Health and Medicine (reference number NCGM-G-001212).

Provenance and peer review

Not commissioned; externally peer reviewed.

Data sharing statement

No additional data are available.

REFERENCES

- 1
2
3
4
5
6 1. Gardner DG, Chen S, Glenn DJ, Grigsby CL. Molecular biology of the natriuretic
7
8 peptide system: implications for physiology and hypertension. *Hypertension*
9
10 2007;49:419-26.
11
- 12
13
14 2. Maeda K, Tsutamoto T, Wada A, et al. High levels of plasma brain natriuretic
15
16 peptide and interleukin-6 after optimized treatment for heart failure are independent risk
17
18 factors for morbidity and mortality in patients with congestive heart failure. *J Am Coll*
19
20 *Cardiol* 2000;36:1587-93.
21
22
- 23
24
25 3. Wang TJ, Larson MG, Levy D, et al. Plasma natriuretic peptide levels and the risk of
26
27 cardiovascular events and death. *N Engl J Med* 2004;350:655-63.
28
29
- 30
31
32 4. Alehagen U, Lindstedt G, Levin LA, Dahlström U. Risk of cardiovascular death in
33
34 elderly patients with possible heart failure. B-type natriuretic peptide (BNP) and the
35
36 aminoterminal fragment of ProBNP (N-terminal proBNP) as prognostic indicators in a
37
38 6-year follow-up of a primary care population. *Int J Cardiol* 2005;100:125-33.
39
40
- 41
42
43 5. Kroon MH, van den Hurk K, Alsema M, et al. Prospective associations of B-type
44
45 natriuretic peptide with markers of left ventricular function in individuals with and
46
47 without type 2 diabetes: an 8-year follow-up of the Hoorn Study. *Diabetes Care*
48
49 2012;35:2510-14.
50
51
- 52
53
54 6. Olsen MH, Wachtell K, Tuxen C, et al. N-terminal pro-brain natriuretic peptide
55
56
57
58
59
60

1
2
3
4
5
6 predicts cardiovascular events in patients with hypertension and left ventricular
7
8 hypertrophy: a LIFE study. *J Hypertens* 2004;22:1597-604.

9
10
11 7. Schirmer H, Omland T. Circulating N-terminal pro-atrial natriuretic peptide is an
12
13 independent predictor of left ventricular hypertrophy in the general population. The
14
15 Tromsø Study. *Eur Heart J* 1999;20:755-63.

16
17
18 8. Wang TJ, Larson MG, Levy D, et al. Impact of obesity on plasma natriuretic peptide
19
20 levels. *Circulation* 2004;109:594-600.

21
22
23 9. Koizumi M, Watanabe H, Kaneko Y, et al. Impact of obesity on plasma B-type
24
25 natriuretic peptide levels in Japanese community-based subjects. *Heart Vessels* 2012;
26
27 27:287-294.

28
29
30 10. Olsen MH, Hansen TW, Christensen MK, et al. N-terminal pro brain natriuretic
31
32 peptide is inversely related to metabolic cardiovascular risk factors and the metabolic
33
34 syndrome. *Hypertension* 2005, 46:660-6.

35
36
37 11. Hamilton MT, Hamilton DG, Zderic TW. Role of low energy expenditure and sitting
38
39 in obesity, metabolic syndrome, type 2 diabetes, and cardiovascular disease. *Diabetes*
40
41 2007;56:2655-67.

42
43
44 12. Avery L, Flynn D, van Wersch A, Sniehotta FF, Trenell MI. Changing physical
45
46 activity behavior in type 2 diabetes: a systematic review and meta-analysis of behavioral
47
48
49
50
51
52
53
54
55
56
57
58
59
60

1
2
3
4
5
6 interventions. *Diabetes Care* 2012;35:2681-89.

7
8
9 13. Barletta G, Stefani L, Del Bene R, et al. Effects of exercise on natriuretic peptides
10
11 and cardiac function in man. *Int J Cardiol* 1998;65:217-25.

12
13
14 14. Nielsen HB, De Palo EF, Meneghetti M, Madsen PL, Ihlemann N, Secher NH.
15
16 Circulating immunoreactive proANP1-30 and proANP31-67 responses to acute exercise.
17
18
19
20
21 *Regul Pept* 2001;99:203-7.

22
23 15. Levine JA. Non-exercise activity thermogenesis (NEAT). *Nutr Rev* 2004;62:S82-97.

24
25
26 16. American Diabetes Association. Diagnosis and classification of diabetes mellitus.
27
28
29
30
31 *Diabetes Care* 2010;33:S62-9.

32
33 17. Oshima Y, Kawaguchi K, Tanaka S, et al. Classifying household and locomotive
34
35 activities using a triaxial accelerometer. *Gait Posture* 2010;31:370-4.

36
37
38 18. Ohkawara K, Oshima Y, Hikiyama Y, Ishikawa-Takata K, Tabata I, Tanaka S.
39
40
41
42
43
44
45 Real-time estimation of daily physical activity intensity by a triaxial accelerometer and
46
47 a gravity-removal classification algorithm. *Br J Nutr* 2011;105:1681-91.

48
49
50 19. Ganpule AA, Tanaka S, Ishikawa-Takata K, Tabata I. Interindividual variability in
51
52
53
54
55
56 sleeping metabolic rate in Japanese subjects. *Eur J Clin Nutr* 2007;61:1256-61.

57
58
59 20. Energy and protein requirements. Report of a joint FAO/WHO/UNU Expert
60
61
62
63
64
65
66
67
68
69
70
71
72
73
74
75
76
77
78
79
80
81
82
83
84
85
86
87
88
89
90
91
92
93
94
95
96
97
98
99
100
101
102
103
104
105
106
107
108
109
110
111
112
113
114
115
116
117
118
119
120
121
122
123
124
125
126
127
128
129
130
131
132
133
134
135
136
137
138
139
140
141
142
143
144
145
146
147
148
149
150
151
152
153
154
155
156
157
158
159
160
161
162
163
164
165
166
167
168
169
170
171
172
173
174
175
176
177
178
179
180
181
182
183
184
185
186
187
188
189
190
191
192
193
194
195
196
197
198
199
200
201
202
203
204
205
206
207
208
209
210
211
212
213
214
215
216
217
218
219
220
221
222
223
224
225
226
227
228
229
230
231
232
233
234
235
236
237
238
239
240
241
242
243
244
245
246
247
248
249
250
251
252
253
254
255
256
257
258
259
260
261
262
263
264
265
266
267
268
269
270
271
272
273
274
275
276
277
278
279
280
281
282
283
284
285
286
287
288
289
290
291
292
293
294
295
296
297
298
299
300
301
302
303
304
305
306
307
308
309
310
311
312
313
314
315
316
317
318
319
320
321
322
323
324
325
326
327
328
329
330
331
332
333
334
335
336
337
338
339
340
341
342
343
344
345
346
347
348
349
350
351
352
353
354
355
356
357
358
359
360
361
362
363
364
365
366
367
368
369
370
371
372
373
374
375
376
377
378
379
380
381
382
383
384
385
386
387
388
389
390
391
392
393
394
395
396
397
398
399
400
401
402
403
404
405
406
407
408
409
410
411
412
413
414
415
416
417
418
419
420
421
422
423
424
425
426
427
428
429
430
431
432
433
434
435
436
437
438
439
440
441
442
443
444
445
446
447
448
449
450
451
452
453
454
455
456
457
458
459
460
461
462
463
464
465
466
467
468
469
470
471
472
473
474
475
476
477
478
479
480
481
482
483
484
485
486
487
488
489
490
491
492
493
494
495
496
497
498
499
500
501
502
503
504
505
506
507
508
509
510
511
512
513
514
515
516
517
518
519
520
521
522
523
524
525
526
527
528
529
530
531
532
533
534
535
536
537
538
539
540
541
542
543
544
545
546
547
548
549
550
551
552
553
554
555
556
557
558
559
560
561
562
563
564
565
566
567
568
569
570
571
572
573
574
575
576
577
578
579
580
581
582
583
584
585
586
587
588
589
590
591
592
593
594
595
596
597
598
599
600
601
602
603
604
605
606
607
608
609
610
611
612
613
614
615
616
617
618
619
620
621
622
623
624
625
626
627
628
629
630
631
632
633
634
635
636
637
638
639
640
641
642
643
644
645
646
647
648
649
650
651
652
653
654
655
656
657
658
659
660
661
662
663
664
665
666
667
668
669
670
671
672
673
674
675
676
677
678
679
680
681
682
683
684
685
686
687
688
689
690
691
692
693
694
695
696
697
698
699
700
701
702
703
704
705
706
707
708
709
710
711
712
713
714
715
716
717
718
719
720
721
722
723
724
725
726
727
728
729
730
731
732
733
734
735
736
737
738
739
740
741
742
743
744
745
746
747
748
749
750
751
752
753
754
755
756
757
758
759
760
761
762
763
764
765
766
767
768
769
770
771
772
773
774
775
776
777
778
779
780
781
782
783
784
785
786
787
788
789
790
791
792
793
794
795
796
797
798
799
800
801
802
803
804
805
806
807
808
809
810
811
812
813
814
815
816
817
818
819
820
821
822
823
824
825
826
827
828
829
830
831
832
833
834
835
836
837
838
839
840
841
842
843
844
845
846
847
848
849
850
851
852
853
854
855
856
857
858
859
860
861
862
863
864
865
866
867
868
869
870
871
872
873
874
875
876
877
878
879
880
881
882
883
884
885
886
887
888
889
890
891
892
893
894
895
896
897
898
899
900
901
902
903
904
905
906
907
908
909
910
911
912
913
914
915
916
917
918
919
920
921
922
923
924
925
926
927
928
929
930
931
932
933
934
935
936
937
938
939
940
941
942
943
944
945
946
947
948
949
950
951
952
953
954
955
956
957
958
959
960
961
962
963
964
965
966
967
968
969
970
971
972
973
974
975
976
977
978
979
980
981
982
983
984
985
986
987
988
989
990
991
992
993
994
995
996
997
998
999
1000

- 1
2
3
4
5
6 21. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC.
7
8 Homeostasis model assessment: insulin resistance and beta-cell function from fasting
9
10 plasma glucose and insulin concentrations in man. *Diabetologia* 1985;28:412-419.
11
12
13
14 22. Mongia SK, La'ulu SL, Apple FS, Ler R, Murakami MM, Roberts WL. Performance
15
16 characteristics of the Architect brain natriuretic peptide (BNP) assay: a two site study.
17
18
19
20
21 *Clin Chim Acta* 2008;391:102-5.
22
23
24 23. Neeland IJ, Winders BR, Ayers CR, et al. Higher natriuretic peptide levels associate
25
26 with a favorable adipose tissue distribution profile. *J Am Coll Cardiol* 2013;62:752-60.
27
28
29 24. Chainani-Wu N, Weidner G, Purnell DM, et al. Relation of B-type natriuretic
30
31 peptide levels to body mass index after comprehensive lifestyle changes. *Am J Cardiol*
32
33
34
35 2010;105:1570-6.
36
37
38 25. Mehra MR, Uber PA, Park MH, et al. Obesity and suppressed B-type natriuretic
39
40 peptide levels in heart failure. *J Am Coll Cardiol* 2004;43:1590-5.
41
42
43 26. McCord J, Mundy BJ, Hudson MP, et al. Relationship between obesity and B-type
44
45 natriuretic peptide levels. *Arch Intern Med* 2004;164:2247-52.
46
47
48
49 27. Dessì-Fulgheri P, Sarzani R, Tamburrini P, et al. Plasma atrial natriuretic peptide and
50
51 natriuretic peptide receptor gene expression in adipose tissue of normotensive and
52
53
54
55 hypertensive obese patients. *J Hypertens* 1997;15:1695-9.
56
57
58
59
60

- 1
2
3
4
5
6 28. Das SR, Drazner MH, Dries DL, et al. Impact of body mass and body composition
7
8 on circulating levels of natriuretic peptides: results from the Dallas Heart Study.
9
10 *Circulation* 2005;112:2163-8.
11
12
13
14 29. Costello-Boerrigter LC, Burnett JC Jr. A new role for the natriuretic peptides:
15
16 metabolic regulators of the adipocyte. *J Am Coll Cardiol* 2009;53:2078-9.
17
18
19
20 30. Sengenès C, Berlan M, De Glisezinski I, Lafontan M, Galitzky J. Natriuretic
21
22 peptides: a new lipolytic pathway in human adipocytes. *FASEB J* 2000;14:1345-51.
23
24
25
26 31. Miyashita K, Itoh H, Tsujimoto H, et al. Natriuretic
27
28 peptides/cGMP/cGMP-dependent protein kinase cascades promote muscle
29
30 mitochondrial biogenesis and prevent obesity. *Diabetes* 2009;58:2880-92.
31
32
33
34 32. Tsukamoto O, Fujita M, Kato M, et al. Natriuretic peptides enhance the production
35
36 of adiponectin in human adipocytes and in patients with chronic heart failure. *J Am Coll*
37
38 *Cardiol* 2009;53:2070-7.
39
40
41
42
43 33. Rajagopalan S, Kurz S, Münzel T, et al. Angiotensin II-mediated hypertension in the
44
45 rat increases vascular superoxide production via membrane NADH/NADPH oxidase
46
47 activation. Contribution to alterations of vasomotor tone. *J Clin Invest*
48
49
50
51 1996;97:1916-23.
52
53
54
55 34. Moro C, Klimcakova E, Lolmède K, et al. Atrial natriuretic peptide inhibits the
56
57
58
59
60

1
2
3
4
5
6 production of adipokines and cytokines linked to inflammation and insulin resistance in
7
8
9 human subcutaneous adipose tissue. *Diabetologia* 2007;50:1038-47.

10
11 35. [Bordicchia M, Liu D, Amri EZ, et al. Cardiac natriuretic peptides act via p38 MAPK](#)
12 [to induce the brown fat thermogenic program in mouse and human adipocytes. *J Clin*](#)
13 [*Invest* 2012;122:1022-36.](#)

14
15
16
17 36. [Engeli S, Birkenfeld AL, Badin PM, et al. Natriuretic peptides enhance the oxidative](#)
18 [capacity of human skeletal muscle. *J Clin Invest* 2012;122:4675-9.](#)

19
20
21 37. [Polak J, Kotrc M, Wedellova Z, et al. Lipolytic effects of B-type natriuretic peptide](#)
22 [1-32 in adipose tissue of heart failure patients compared with healthy controls. *J Am*](#)
23 [*Coll Cardiol* 2011;58:1119-25.](#)

24
25
26 38. [Tekes S, Cikim AS. The association of brain natriuretic peptide and insulin](#)
27 [resistance in obesity-related hypertension. *J Hum Hypertens* 2007;21:546-50.](#)

28
29
30 39. Plasqui G, Bonomi AG, Westerterp KR. Daily physical activity assessment with
31 [accelerometers: new insights and validation studies. *Obes Rev* 2013;14:451-62.](#)

32
33
34 40. Midorikawa T, Tanaka S, Kaneko K, et al. Evaluation of low-intensity physical
35 [activity by triaxial accelerometry. *Obesity* 2007;15:3031-8.](#)

36
37
38 41. Ekelund U, Sjöström M, Yngve A, et al. Physical activity assessed by activity
39 [monitor and doubly labeled water in children. *Med Sci Sports Exerc* 2001;33:275-81.](#)

1
2
3
4
5
6 42. Leenders NY, Sherman WM, Nagaraja HN. Energy expenditure estimated by
7
8
9 accelerometry and doubly labeled water: do they agree? *Med Sci Sports Exerc*
10
11
12 2006;38:2165-72.
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

For peer review only

(figure legends)

Figure 1 Correlation of physical activity level and plasma BNP levels in 60 subjects.

r indicates correlation coefficient analyzed by the Spearman's rank correlation coefficient.

Figure 2 Serum insulin levels (A) and homeostatic model assessment-estimated insulin resistance (HOMA-IR) (B) in high-BNP group (n = 30) and low-BNP group (n = 30).

Data are mean values±SD. We divided subjects into high-BNP group (≥ 8.0 pg/ml) and low-BNP group (< 8.0 pg/ml) by the median values of BNP. Differences in serum insulin levels and HOMA-IR between high-BNP group and low-BNP group were analyzed by the Mann-Whitney U test.

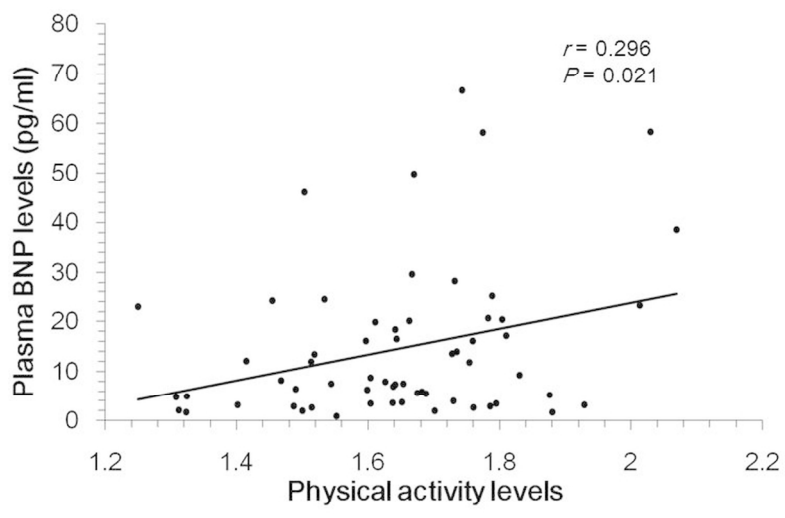
Figure 3 Serum insulin levels (A) and homeostatic model assessment-estimated insulin resistance (HOMA-IR) (B) in each plasma BNP quartiles. Q1 is the lowest quartile and

Q4 is the highest quartile. Boxes indicate medians and quartiles; whiskers indicate the lowest and highest values. We found a significant influence of plasma BNP on both serum insulin (p=0.018 by the Kruskal-Wallis ANOVA) and HOMA-IR (p=0.018). The difference between Q1 and Q2-4 was statistically analyzed by the Scheffe's F Test.

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

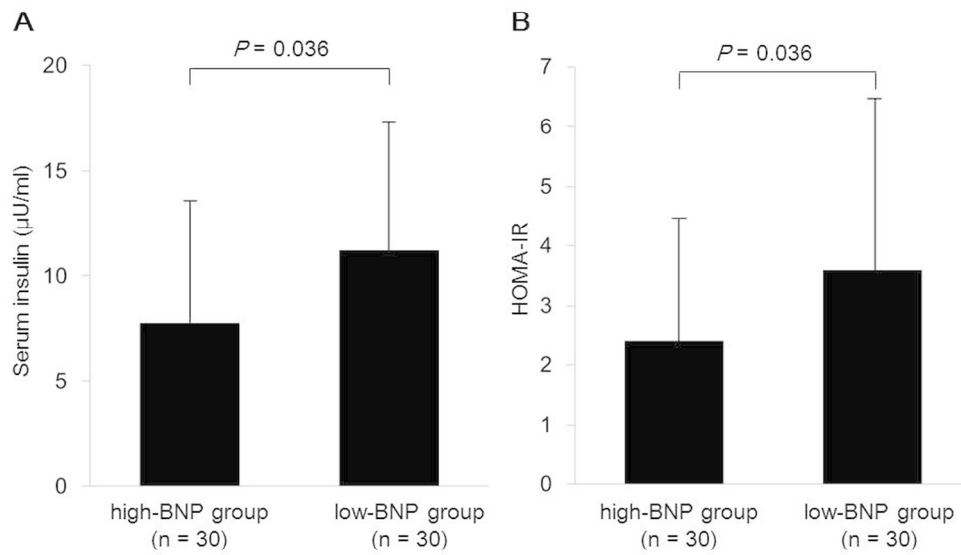
For peer review only

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60



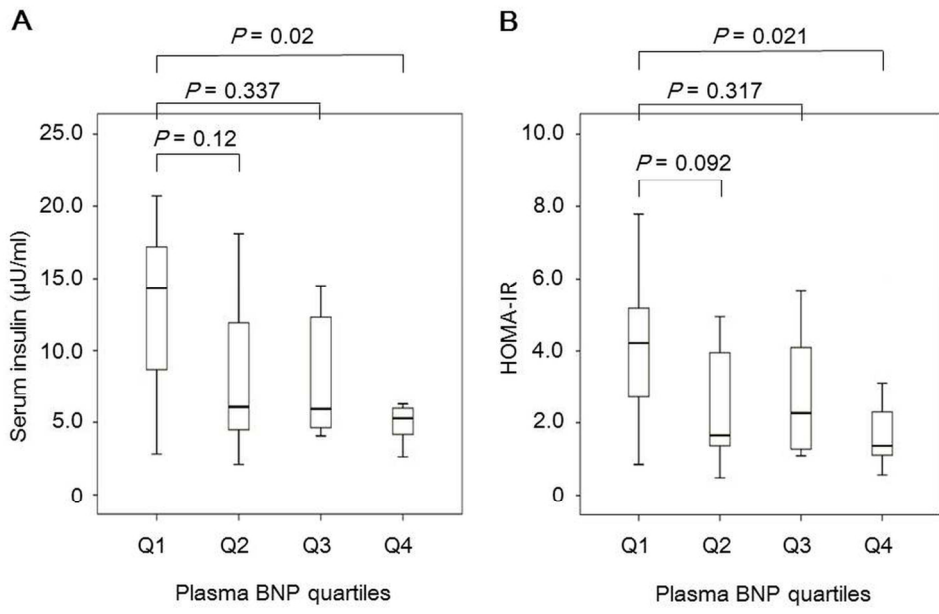
119x90mm (300 x 300 DPI)

ew only



119x90mm (300 x 300 DPI)

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60



119x90mm (300 x 300 DPI)

Review only

BMJ Open

The association between daily physical activity and plasma B-type natriuretic peptide in patients with glucose intolerance: a cross-sectional study

Journal:	<i>BMJ Open</i>
Manuscript ID:	bmjopen-2014-006276.R2
Article Type:	Research
Date Submitted by the Author:	16-Dec-2014
Complete List of Authors:	Hamasaki, Hidetaka; National Center for Global Health and Medicine Kohnodai Hospital, Department of Internal Medicine Yanai, Hidekatsu; National Center for Global Health and Medicine Kohnodai Hospital, Department of Internal Medicine Kakei, Masafumi; Jichi Medical University School of Medicine, Noda, Mitsuhiro; Center Hospital, National Center for Global Health and Medicine, Ezaki, Osamu; Showa Women's University,
Primary Subject Heading:	Diabetes and endocrinology
Secondary Subject Heading:	Diabetes and endocrinology
Keywords:	DIABETES & ENDOCRINOLOGY, SPORTS MEDICINE, PUBLIC HEALTH

SCHOLARONE™
Manuscripts

Research**The association between daily physical activity and plasma B-type natriuretic peptide in patients with glucose intolerance: a cross-sectional study**

Hidetaka Hamasaki,^{1,2} Hidekatsu Yanai,¹ Masafumi Kakei,² Mitsuhiro Noda,³ Osamu Ezaki⁴

¹ Department of Internal Medicine, National Center for Global Health and Medicine Kohnodai Hospital, Chiba, Japan.

² Division of Complementary Medicine, First Department of General Medicine, Saitama Medical Center, Jichi Medical University School of Medicine, Saitama, Japan.

³ Department of Diabetes and Metabolic Medicine, Center Hospital, National Center for Global Health and Medicine, Tokyo, Japan.

⁴ Department of Human Health and Design, Faculty of Human Life and Environmental Sciences, Showa Women's University, Tokyo, Japan.

Correspondence to

1
2
3
4
5
6 Hidekatsu Yanai, MD, PhD, FACP, Department of Internal Medicine, National Center
7
8
9 for Global Health and Medicine Kohnodai Hospital, 1-7-1 Kohnodai, Chiba 272-8516,
10
11 Japan. E-mail: dyanai@hospk.ncgm.go.jp; Telephone: +81-47-372-3501; Fax:
12
13 +81-47-372-1858
14
15
16
17
18
19
20
21
22

23 **Keywords:** B-type natriuretic peptide; glucose intolerance; insulin resistance; physical
24
25 activity; type 2 diabetes
26
27
28
29
30
31
32

33 **Word count:** 2,732 words
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

ABSTRACT

Objectives: In spite of accumulating evidences suggesting an inverse association between insulin resistance and plasma B-type natriuretic peptide (BNP) levels, the effect of daily physical activity on plasma BNP in individuals with glucose intolerance remains unknown. We investigated the association of physical activity level (PAL) with plasma BNP in patients with impaired fasting glucose, impaired glucose tolerance and type 2 diabetes.

Design: Cross-sectional study.

Setting: Outpatients visiting National Center for Global Health and Medicine Kohnodai Hospital.

Participants: A total of 60 patients with glucose intolerance who did not take any hypoglycemic agents, cholesterol lowering agents and antihypertensive agents were recruited. Patients who were diagnosed as having heart failure and renal impairment, engaged in sports-like exercise and resistance training were excluded.

Primary outcome measures: PAL was objectively measured by a triaxial accelerometer. The association between PAL and plasma BNP levels was assessed by multiple regression analysis.

Results: PAL was positively correlated with plasma BNP levels ($r=0.296$, $p=0.021$).

1
2
3
4
5
6 PAL was still significantly correlated with plasma BNP levels after adjustment for age
7
8
9 ($\beta=0.290$, $p=0.014$), and adjustment for age and BMI ($\beta=0.282$, $p=0.018$). Plasma BNP
10
11
12 levels were inversely correlated with serum insulin levels ($r=-0.350$, $p=0.006$) and
13
14 homeostasis model assessment-estimated insulin resistance (HOMA-IR) ($r=-0.363$,
15
16 $p=0.004$). Serum insulin levels (mean \pm SD, 8.1 ± 6.4 $\mu\text{U/ml}$) and HOMA-IR ($2.4 \pm$
17
18 1.9) in high-BNP group were significantly lower than those (11.2 ± 7.4 $\mu\text{U/ml}$ and $3.7 \pm$
19
20 3.0 , respectively) in low-BNP group.
21
22
23
24

25
26 **Conclusions:** Our findings propose the possibility that plasma BNP may be increased
27
28
29 by daily physical activity and BNP is associated with insulin resistance.
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

Strengths and limitations of this study

- This study provides novel data: objectively measured light-intensity daily physical activity using a triaxial accelerometer is positively associated with plasma BNP levels in patients with glucose intolerance.
- Our study evaluated precisely an association between daily physical activity and plasma BNP levels, by recruiting drug-naive patients who were not engaged in sports-like exercise.
- Previous studies reported an inverse association between plasma BNP levels and the markers for insulin resistance in healthy subjects. To our knowledge, our study is the first to show a significant association between plasma BNP levels and insulin resistance in patients with glucose intolerance.
- The limitations are the small sample size and the cross-sectional design which does not allow us to establish any causal relationship.
- Even a triaxial accelerometer may lead to under or overestimation of energy expenditure. It can be denied that energy expenditure assessed by a triaxial accelerometer differ from the true amounts.

INTRODUCTION

B-type natriuretic peptide (BNP) belongs to the cardiac natriuretic peptide family which are released from the heart in response to pressure and volume overload.¹ Plasma BNP and N-terminal proBNP (Nt-proBNP) are increased with the severity of left ventricular dysfunction and/or hypertrophy, therefore, it has become a useful biomarker for diagnosis and prognosis of heart failure with or without type 2 diabetes.²⁻⁷

Plasma BNP levels have been reported to be inversely related to body mass index (BMI) and waist circumference in individuals without heart failure.⁸⁻⁹ Very recently, Olsen MH, et al. reported that Nt-proBNP was significantly lower in individuals with the metabolic syndrome as compared with those without the metabolic syndrome.¹⁰ In their study, Nt-proBNP levels were inversely correlated with BMI, waist circumference, serum cholesterol and triglyceride, plasma glucose and insulin, independently of age and gender.¹⁰ These results suggest that BNP is beneficially associated with obesity and obesity-related metabolic disorders.

Low physical activity and sedentary lifestyle induce the development of type 2 diabetes, and also deteriorate glucose control in patients with type 2 diabetes.¹¹⁻¹² Exercise such as bicycle and hand-grip exercise has been reported to acutely increase plasma BNP levels with exercise intensity, in healthy individuals.¹³⁻¹⁴ However, the

1
2
3
4
5
6 effect of daily physical activity on plasma BNP levels remain obscure. To our
7
8
9 knowledge, there were no previous studies that investigated the association between
10
11
12 daily physical activity and plasma BNP levels in patients with glucose intolerance.
13

14
15 Here, we studied the association of physical activity level (PAL) which was
16
17 evaluated objectively by using a triaxial accelerometer, with plasma BNP levels, in
18
19 patients with impaired fasting glucose (IFG), impaired glucose tolerance (IGT) and type
20
21 2 diabetes. Furthermore, we elucidated the correlations of plasma BNP levels to
22
23 metabolic parameters in patients with such glucose intolerance.
24
25
26
27
28
29
30
31

32 **MATERIAL AND METHODS**

33 34 **Study protocol and participants**

35
36
37 The study protocol was approved by the Medical Ethics Committee of National Center
38
39 for Global Health and Medicine (reference number NCGM-G-001212). We recruited
40
41 study participants with glucose intolerance who did not take any hypoglycemic agents
42
43 or cholesterol-lowering agents from outpatients who visited the Department of Internal
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65
66
67
68
69
70
71
72
73
74
75
76
77
78
79
80
81
82
83
84
85
86
87
88
89
90
91
92
93
94
95
96
97
98
99
100
101
102
103
104
105
106
107
108
109
110
111
112
113
114
115
116
117
118
119
120
121
122
123
124
125
126
127
128
129
130
131
132
133
134
135
136
137
138
139
140
141
142
143
144
145
146
147
148
149
150
151
152
153
154
155
156
157
158
159
160
161
162
163
164
165
166
167
168
169
170
171
172
173
174
175
176
177
178
179
180
181
182
183
184
185
186
187
188
189
190
191
192
193
194
195
196
197
198
199
200
201
202
203
204
205
206
207
208
209
210
211
212
213
214
215
216
217
218
219
220
221
222
223
224
225
226
227
228
229
230
231
232
233
234
235
236
237
238
239
240
241
242
243
244
245
246
247
248
249
250
251
252
253
254
255
256
257
258
259
260
261
262
263
264
265
266
267
268
269
270
271
272
273
274
275
276
277
278
279
280
281
282
283
284
285
286
287
288
289
290
291
292
293
294
295
296
297
298
299
300
301
302
303
304
305
306
307
308
309
310
311
312
313
314
315
316
317
318
319
320
321
322
323
324
325
326
327
328
329
330
331
332
333
334
335
336
337
338
339
340
341
342
343
344
345
346
347
348
349
350
351
352
353
354
355
356
357
358
359
360
361
362
363
364
365
366
367
368
369
370
371
372
373
374
375
376
377
378
379
380
381
382
383
384
385
386
387
388
389
390
391
392
393
394
395
396
397
398
399
400
401
402
403
404
405
406
407
408
409
410
411
412
413
414
415
416
417
418
419
420
421
422
423
424
425
426
427
428
429
430
431
432
433
434
435
436
437
438
439
440
441
442
443
444
445
446
447
448
449
450
451
452
453
454
455
456
457
458
459
460
461
462
463
464
465
466
467
468
469
470
471
472
473
474
475
476
477
478
479
480
481
482
483
484
485
486
487
488
489
490
491
492
493
494
495
496
497
498
499
500
501
502
503
504
505
506
507
508
509
510
511
512
513
514
515
516
517
518
519
520
521
522
523
524
525
526
527
528
529
530
531
532
533
534
535
536
537
538
539
540
541
542
543
544
545
546
547
548
549
550
551
552
553
554
555
556
557
558
559
560
561
562
563
564
565
566
567
568
569
570
571
572
573
574
575
576
577
578
579
580
581
582
583
584
585
586
587
588
589
590
591
592
593
594
595
596
597
598
599
600
601
602
603
604
605
606
607
608
609
610
611
612
613
614
615
616
617
618
619
620
621
622
623
624
625
626
627
628
629
630
631
632
633
634
635
636
637
638
639
640
641
642
643
644
645
646
647
648
649
650
651
652
653
654
655
656
657
658
659
660
661
662
663
664
665
666
667
668
669
670
671
672
673
674
675
676
677
678
679
680
681
682
683
684
685
686
687
688
689
690
691
692
693
694
695
696
697
698
699
700
701
702
703
704
705
706
707
708
709
710
711
712
713
714
715
716
717
718
719
720
721
722
723
724
725
726
727
728
729
730
731
732
733
734
735
736
737
738
739
740
741
742
743
744
745
746
747
748
749
750
751
752
753
754
755
756
757
758
759
760
761
762
763
764
765
766
767
768
769
770
771
772
773
774
775
776
777
778
779
780
781
782
783
784
785
786
787
788
789
790
791
792
793
794
795
796
797
798
799
800
801
802
803
804
805
806
807
808
809
810
811
812
813
814
815
816
817
818
819
820
821
822
823
824
825
826
827
828
829
830
831
832
833
834
835
836
837
838
839
840
841
842
843
844
845
846
847
848
849
850
851
852
853
854
855
856
857
858
859
860
861
862
863
864
865
866
867
868
869
870
871
872
873
874
875
876
877
878
879
880
881
882
883
884
885
886
887
888
889
890
891
892
893
894
895
896
897
898
899
900
901
902
903
904
905
906
907
908
909
910
911
912
913
914
915
916
917
918
919
920
921
922
923
924
925
926
927
928
929
930
931
932
933
934
935
936
937
938
939
940
941
942
943
944
945
946
947
948
949
950
951
952
953
954
955
956
957
958
959
960
961
962
963
964
965
966
967
968
969
970
971
972
973
974
975
976
977
978
979
980
981
982
983
984
985
986
987
988
989
990
991
992
993
994
995
996
997
998
999
1000

1
2
3
4
5
6 having type 2 diabetes, IFG and IGT, by performing the 75 g oral glucose tolerance test
7
8
9 (OGTT) and the measurement of hemoglobin A1c (HbA1c), fasting plasma glucose
10
11
12 (FPG), casual plasma glucose and 2-h values in the OGTT. The participants were
13
14
15 diagnosed as having type 2 diabetes according to diagnostic criteria set to serum levels
16
17 of HbA1c \geq 6.5 %, FPG \geq 126 mg/dl, casual plasma glucose \geq 200 mg/dl and
18
19
20 2-h values in the OGTT \geq 200 mg/dl.¹⁵ The participants were defined as having IFG
21
22
23 and IGT according to FPG levels 110 mg/dl to 125 mg/dl, or 2-h values in the OGTT of
24
25
26 140 mg/dl to 199 mg/dl.¹⁵ To understand the effects of daily physical activity such as
27
28
29 non-exercise activity thermogenesis (NEAT)¹⁶ on BNP and metabolic parameters,
30
31
32 participants who were engaged in sports-like exercise and resistance training were
33
34
35 excluded (exclusion criteria). Participants with heart failure and renal impairment were
36
37
38 also excluded (exclusion criteria).
39
40
41
42
43

44 **Anthropometric and physiological measurements**

45
46 Height and weight were measured using a rigid stadiometer and scales (DP-7100PW,
47
48 Yamato Co., Ltd, Hyogo, Japan). Waist circumference around the navel was measured
49
50
51 with a metal anthropometric tape while participants are standing and breathing out. BMI
52
53
54 was calculated as the body weight in kilograms divided by the height in meters squared.
55
56
57
58
59
60

1
2
3
4
5
6 Blood pressure was measured with participants in a seated position using an automatic
7
8 sphygmomanometer (HEM-762, Omron Co., Ltd, Kyoto, Japan).
9
10

11 12 13 14 15 **Physical activity measurement** 16

17
18 Daily physical activity was measured using a triaxial accelerometer (Active Style Pro
19
20 HJA-350IT, Omron Co., Ltd, Kyoto, Japan), 74 x 46 x 34 mm and 60 g including
21
22 batteries. Participants studied wore the accelerometer on the left side of the waist.
23
24 Anteroposterior, mediolateral and vertical acceleration measurements were obtained
25
26 during each physical activity at a rate of 32 Hz to 12-bit accuracy. Each of three signals
27
28 from the triaxial accelerometer was passed through a high-pass filter with a cut-off
29
30 frequency of 0.7 Hz to remove the gravitational acceleration component. The ratios of
31
32 unfiltered to filtered total acceleration (TAU/TAF) and filtered vertical and horizontal
33
34 acceleration (VAF/HAF) were calculated to determine the cut-off value for the
35
36 classification of locomotive activities and non-locomotive activities including such as
37
38 household and occupational activities, which resulted in almost 100% accurate
39
40 demarcation for daily eleven different activities.¹⁷ Furthermore, metabolic equivalent
41
42 values (METs) determined by this triaxial accelerometer have been reported to be
43
44 closely correlated with METs calculated by using energy expenditure (EE) measured by
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

1
2
3
4
5
6 indirect calorimetry.^{17 18}
7
8

9 Participants studied wore the accelerometer on the left side of the waist for
10 consecutive 7 days, and physical activities were recorded. Participants were requested to
11 wear the accelerometer except under special circumstances such as sleeping, bathing
12 and during aquatic activities. Activity data were stored on a minute-by-minute basis and
13 were downloaded to a personal computer before analysis. We excluded days in which
14 participants did not wear the accelerometer for more than 8 hours from the data for
15 analysis.
16
17
18
19
20
21
22
23
24
25
26
27
28

29 Basal metabolic rate (BMR) was estimated from multiple regression equation
30 including age, sex, height and ideal body weight (IBW) as variables, the equation as
31 follows: $BMR \text{ (kcal/day)} = [(0.1283 + 0.0481 \times IBW \text{ (kg)} + 0.0234 \times \text{height (cm)} -$
32 $0.0138 \times \text{age (year)} - 0.5473 \times \text{sex coefficient (man: 1, woman:2)}) \times 293]$.¹⁹ Total
33 energy expenditure (TEE) was calculated by manufactured regression equation using
34 METs assessed by the triaxial accelerometer.¹⁸ Physical activity level (PAL) was
35 calculated by the following equation. $PAL = TEE / BMR$.²⁰
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51

52 **Blood examination, BNP measurements**

53
54

55 After a 12-h overnight fast, blood samples were taken from the antecubital vein and
56
57
58
59
60

1
2
3
4
5
6 collected into tubes. We measured FPG, HbA1c and serum insulin. FPG was measured
7
8
9 using an enzymatic method (Wako Pure Chemical Industries, Osaka, Japan). Serum
10
11
12 insulin and HbA1c were measured by automated enzyme-linked immunosorbent assays
13
14 (TOSOH, Tokyo, Japan). We used homeostasis model assessment-estimated insulin
15
16 resistance (HOMA-IR) as the marker for insulin resistance.²¹ Plasma BNP (BNP 1-32)
17
18 levels were measured using specific immunoradiometric assay for human BNP
19
20 (ARCHITECT BNP-JP[®], ABBOTT JAPAN Co., Ltd, Tokyo, Japan).²² Imprecision
21
22 studies yielded within run CVs of 1.1 to 5.1% and total CVs of 2.3 to 5.3% using human
23
24 plasma based multi-constituent controls at concentrations of 92, 500, and 3500 ng/L.²²
25
26
27
28
29
30
31
32
33
34

35 **Statistical analysis**

36
37
38 Data were expressed as the mean \pm standard deviation (SD). Correlations among BNP,
39
40 age, BMI, waist circumference, blood pressure, FPG, HbA1c, serum insulin levels,
41
42 HOMA-IR and PAL were assessed by using the Spearman's rank correlation coefficient.
43
44 Multiple regression analysis was performed to test the independent correlation of PAL
45
46 with plasma BNP levels. We divided participants into high-BNP group (≥ 8.0 pg/ml)
47
48 and low-BNP group (< 8.0 pg/ml) by the median values of BNP. Differences in serum
49
50 insulin levels, HOMA-IR and PAL between high-BNP group and low-BNP group were
51
52
53
54
55
56
57
58
59
60

analyzed by the Mann-Whitney U test. The statistical analyses were performed by using SPSS version 19 (IBM Co., Ltd, Chicago, USA). P value <0.05 was considered to be statistically significant.

RESULTS

We recruited 60 participants (28 men and 32 women) by the inclusion and exclusion criteria for participation in this study. **Table 1** presents the clinical characteristics of studied participants. Thirty one participants were diagnosed as having type 2 diabetes, 14 participants were with IFG and 15 participants were with IGT. The range of age in participants studied was 27-74 years old.

Table 1 Clinical and biochemical characteristics of subjects

n	60
Age (years)	54.7 ± 12.2
Height (cm)	161.3 ± 8.9
Weight (kg)	69.2 ± 16.1
BMI (kg/m ²)	26.5 ± 5.1
Waist circumference (cm)	92.4 ± 13.1
Systolic blood pressure (mmHg)	128.8 ± 17.6

1		
2		
3		
4		
5		
6	Diastolic blood pressure (mmHg)	80.5 ± 12.5
7		
8		
9	Fasting plasma glucose (mg/dl)	122.9 ± 25.6
10		
11		
12	HbA1c (%)	6.7 ± 1.1
13		
14		
15	BNP (pg/ml)	14.0 ± 14.6
16		
17		
18	Serum insulin (μU/ml)	9.6 ± 6.9
19		
20		
21	HOMA-IR	3.02 ± 2.62
22	<hr/>	

23
24 Data are expressed as the mean ± SD. BMI, body mass index; BNP, B-type
25
26 natriuretic peptide; HOMA-IR, homeostasis model assessment-estimated
27
28 insulin resistance.
29

30
31
32 PAL evaluated by a triaxial accelerometer was significantly and positively
33
34 correlated with plasma BNP levels ($r=0.296$, $p=0.021$; **Figure 1**). Age was correlated
35
36 with BMI ($r=-0.431$, $p<0.001$), but not correlated with PAL ($r=0.096$, $p=0.462$). BMI
37
38 was not correlated with PAL ($r=-0.11$, $p=0.4$). The values of PAL showed a normal
39
40 distribution ($p=0.453$ by Shapiro-Wilk test), however, plasma BNP levels showed
41
42 non-normal distribution ($p<0.001$). Multivariate logistic regression was used after
43
44 controlling simultaneously for potential confounders. Variables considered in the
45
46 models were age (continuous) and BMI (continuous). To adjust the correlation between
47
48 PAL and plasma BNP by age and BMI, plasma BNP values were logarithmically
49
50
51
52
53
54
55
56
57
58
59
60

transformed. PAL was still significantly correlated with log plasma BNP levels after adjustment for age ($\beta=0.290$, $p=0.014$), and adjustment for age and BMI ($\beta=0.282$, $p=0.018$).

Table 2 shows the correlations of plasma BNP levels to clinical and metabolic parameters in participants.

Table 2 Correlations of plasma BNP levels to clinical and metabolic parameters

	correlation coefficient	<i>P</i> Value
Age (years)	0.441	<0.001
BMI (kg/m ²)	-0.256	0.048
Waist circumference (cm)	-0.279	0.031
Systolic blood pressure (mmHg)	0.175	0.185
Diastolic blood pressure (mmHg)	-0.070	0.598
Fasting plasma glucose (mg/dl)	-0.237	0.068
HbA1c (%)	0.050	0.705
Serum insulin (μ U/ml)	-0.350	0.006

1
2
3
4
5
6 HOMA-IR -0.363 0.004
7
8

9 BMI, body mass index; BNP, B-type natriuretic peptide; HOMA-IR, homeostasis model
10 assessment-estimated insulin resistance. A statistical analysis was performed by the
11 Spearman's rank correlation coefficient.
12
13
14
15
16
17
18
19

20
21 There were no significant correlations of plasma BNP levels with systolic and
22 diastolic blood pressure, FPG and HbA1c. Plasma BNP levels were significantly and
23 inversely correlated with BMI, waist circumference, serum insulin levels and
24 HOMA-IR, and were positively correlated with age. Significant correlations of plasma
25 BNP levels with serum insulin ($\beta=-0.204$, $p=0.119$) and HOMA-IR ($\beta=-0.271$,
26 $p=0.129$) were lost after adjusting for PAL.
27
28
29
30
31
32
33
34
35
36
37

38 To further understand the associations of plasma BNP levels with insulin
39 resistance, we examined the differences in serum insulin levels and HOMA-IR between
40 high-BNP group and low-BNP group (**Figure 2**). Serum insulin levels (mean \pm SD, 8.1
41 ± 6.4 U/ml) in high-BNP group were significantly lower than those (11.2 ± 7.4 U/ml) in
42 low-BNP group. HOMA-IR (2.4 ± 1.9) in high-BNP group were also significantly lower
43 than those (3.7 ± 3.0) in low-BNP group. We also analyzed the relationship between
44 quartiles of plasma BNP versus serum insulin and HOMA-IR (**Figure 3**). We found a
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

1
2
3
4
5
6 significant influence of plasma BNP on both serum insulin ($p=0.018$ by the
7
8 Kruskal-Wallis ANOVA) and HOMA-IR ($p=0.018$). Serum insulin level in the highest
9
10 plasma BNP quartile was significantly lower than in the lowest plasma BNP quartile.
11
12
13 HOMA-IR in the highest plasma BNP quartile was also significantly lower than in the
14
15
16 lowest plasma BNP quartile.
17
18
19

20
21 The mean \pm SD of plasma BNP levels in patients with type 2 diabetes (13 men
22
23 and 18 women), IFG (5 men and 9 women) and IGT (10 men and 5 women) were 14.3
24
25 ± 13.5 pg/ml, 12.9 ± 17.2 pg/ml and 14.5 ± 15.1 pg/ml, respectively. The mean \pm SD of
26
27
28 PAL in patients with type 2 diabetes, IFG and IGT were 1.66 ± 0.22 , 1.65 ± 0.15 and
29
30
31 1.66 ± 0.20 , respectively. There were no significant differences in plasma BNP levels
32
33
34 and PAL among patients with type 2 diabetes, IFG and IGT.
35
36
37
38
39

40 41 **DISCUSSION**

42
43 Recent cross-sectional studies reported that plasma BNP or NT-proBNP levels were
44
45
46 inversely associated with visceral fat,²³ BMI, waist circumference and serum insulin
47
48
49 levels.^{9 10} However, the effect of daily physical activity on plasma BNP levels remains
50
51
52 unknown. Previous cross-sectional studies have not ever been performed by using
53
54
55 individuals with glucose intolerance as our study participants. In the present study, we
56
57
58
59
60

1
2
3
4
5
6 examined the correlation of daily physical activity measured objectively by a triaxial
7
8 accelerometer with plasma BNP levels, in patients with prediabetes and early untreated
9
10 type 2 diabetes.
11
12

13
14 Present study demonstrates a significant and positive association between daily
15
16 physical activity and plasma BNP levels, and also an inverse association of plasma BNP
17
18 levels with BMI, waist circumference, serum insulin levels and HOMA-IR. Previous
19
20 studies reported that exercise such as bicycle and hand-grip exercise acutely increase
21
22 plasma BNP levels with exercise intensity in healthy individuals.^{13 14} However, the
23
24 effect of daily physical activity on plasma BNP levels remains unknown. It is
25
26 noteworthy that we could observe a significant effect of low-intensity daily physical
27
28 activity, including walking, dishing and washing clothes, defined as NEAT,¹⁵ on plasma
29
30 BNP levels. Chainani-Wu N, et al. previously conducted a nested prospective cohort
31
32 study of a lifestyle intervention, to understand the effects of lifestyle on BNP. They
33
34 observed the change in BNP for 3 months was significantly associated with the change
35
36 in exercise score, even after controlling the percentage of change in BMI, which agreed
37
38 with a significant and positive correlation between PAL and plasma BNP levels after
39
40 adjustment by age and BMI in our study. At 3 months in their study, they found an
41
42 inverse association of the change in BNP with the changes in BMI and insulin,²⁴
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

1
2
3
4
5
6 suggesting a significant association between BNP and insulin resistance.
7

8
9 An inverse association between natriuretic peptide and obesity and/or insulin
10 resistance has been established.^{8-10 23 25 26} However, controversy exists as to this
11 association. Lower natriuretic peptide levels in obese individuals has been reported to
12 be due to increased clearance by the natriuretic peptide receptors (NPR)-C abundantly
13 expressed in adipose tissue, and also due to decreased natriuretic peptide release from
14 the heart.²⁷⁻²⁹ BNP binds to NPR on adipose tissue and stimulate lipolysis,³⁰ regulates
15 adipose tissue by activation of proliferator-activated receptor gamma (PPAR γ) gene
16 expression³¹ and increases adiponectin secretion,³² which improves insulin resistance.
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31

32 Evidences for the association of glucose intolerance with BNP are very limited. A
33 previous study reported that diabetes was significantly associated with low plasma BNP
34 levels (adjusted OR 1.51 (1.00 to 2.27) for men; adjusted OR 1.95 (1.26 to 3.02) for
35 women).⁸ However, the underlying mechanisms for low BNP levels in patients with
36 impaired glucose metabolism remain unknown. In present study, PAL was positively
37 correlated with plasma BNP levels, which was inversely correlated with the marker for
38 insulin resistance. Furthermore, serum insulin levels and HOMA-IR were significantly
39 lower in high-BNP group as compared with those in low-BNP group. BMI and waist
40 circumference were also correlated with plasma BNP levels. Increased PAL may be
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

1
2
3
4
5
6 associated with reduction of BMI and waist circumference, which improves insulin
7
8
9 resistance and induce elevation of plasma BNP, due to decreased clearance by NPR-C
10
11
12 abundantly expressed in adipose tissue. Otherwise, increased plasma BNP may
13
14
15 ameliorate insulin resistance through various mechanisms, such as reduced oxidative
16
17
18 stress,³³ decreased systemic inflammation,³⁴ stimulated lipolysis,³⁰ activation of PPAR γ
19
20
21 gene expression and increased adiponectin secretion.^{31 32} Recently, increased plasma
22
23
24 BNP levels and/or biological activity could also improve insulin resistance by
25
26
27 increasing mitochondrial fat oxidative capacity in white and brown fat, as well as in
28
29
30 skeletal muscle.^{35 36} However, the cardiac ejection fraction is also inversely related
31
32
33 with plasma BNP levels but is not beneficial,²⁻⁷ and plasma BNP levels are reported to
34
35
36 increase lipolysis,³⁷ and to be positively associated insulin resistance.³⁸ The
37
38
39 relationships among PAL, BNP and insulin resistance in impaired glucose metabolism
40
41
42 remain largely unknown. We should perform further studies, preferably using a greater
43
44
45 number of patients with glucose intolerance.

46
47
48 There are several limitations that need to be considered when interpreting the
49
50
51 results of this study. This is a cross-sectional study, limiting inferences of causality and
52
53
54 its direction. Although we controlled for some confounding factors (age, sex, BMI,
55
56
57 engagement in sports-like exercise or resistance training, and medication), other factors

1
2
3
4
5
6 such as genetic variation were not taken into account. Furthermore, we studied only
7
8
9 patients with IFG, IGT and type 2 diabetes, therefore, our conclusions cannot apply to
10
11
12 healthy populations. A heterogeneous group of individuals with IFG, IGT and type 2
13
14
15 diabetes was examined. We could not mention that the associations between BNP and
16
17
18 physical activity or insulin resistance were the same in three groups. We will study these
19
20
21 associations, by using a great number of patients with each type of glucose intolerance,
22
23
24 in the future. The triaxial accelerometer is an extensively validated device for evaluating
25
26
27 physical activity under free living conditions,³⁹⁻⁴¹ however, Leenders et al indicated that
28
29
30 the predictive equations based on the relationship between acceleration and energy
31
32
33 expenditure (EE) during locomotive movements led to under- and overestimation of
34
35
36 TEE.⁴² It is possible that EE and PAL assessed by a triaxial accelerometer differ from
37
38
39 the true amounts of EE and PAL.

40
41 In conclusion, we found a significant and positive association between PAL and
42
43
44 plasma BNP levels in patients with prediabetes and early untreated type 2 diabetes.
45
46
47 Plasma BNP levels were inversely associated with insulin resistance. Our findings
48
49
50 propose the possibility that BNP could be increased by daily physical activity and
51
52
53 plasma BNP is beneficially associated with insulin resistance.
54
55
56
57
58
59
60

Contributors

The study was carried out in collaboration between all authors. HH, HY and OE conceived and designed the study. HH and MN performed the study. HH, HY and MK analyzed the data, interpreted the results and wrote the manuscript. HH and HY also discussed analyses, interpretation, presentation and participated in drafting the manuscript.

Funding

This study was supported by a grant from the National Center for Global Health and Medicine (25-203).

Competing interests

The authors declare that there are no conflicts of interest.

Ethics approval

The study was approved by the Medical Ethics Committee of National Center for Global Health and Medicine (reference number NCGM-G-001212).

Provenance and peer review

Not commissioned; externally peer reviewed.

Data sharing statement

No additional data are available.

REFERENCES

1. Gardner DG, Chen S, Glenn DJ, Grigsby CL. Molecular biology of the natriuretic peptide system: implications for physiology and hypertension. *Hypertension* 2007;49:419-26.
2. Maeda K, Tsutamoto T, Wada A, et al. High levels of plasma brain natriuretic peptide and interleukin-6 after optimized treatment for heart failure are independent risk factors for morbidity and mortality in patients with congestive heart failure. *J Am Coll Cardiol* 2000;36:1587-93.
3. Wang TJ, Larson MG, Levy D, et al. Plasma natriuretic peptide levels and the risk of cardiovascular events and death. *N Engl J Med* 2004;350:655-63.
4. Alehagen U, Lindstedt G, Levin LA, Dahlström U. Risk of cardiovascular death in elderly patients with possible heart failure. B-type natriuretic peptide (BNP) and the

1
2
3
4
5
6 aminoterminal fragment of ProBNP (N-terminal proBNP) as prognostic indicators in a
7
8
9 6-year follow-up of a primary care population. *Int J Cardiol* 2005;100:125-33.

10
11
12 5. Kroon MH, van den Hurk K, Alsema M, et al. Prospective associations of B-type
13
14 natriuretic peptide with markers of left ventricular function in individuals with and
15
16 without type 2 diabetes: an 8-year follow-up of the Hoorn Study. *Diabetes Care*
17
18 2012;35:2510-14.
19
20
21

22
23
24 6. Olsen MH, Wachtell K, Tuxen C, et al. N-terminal pro-brain natriuretic peptide
25
26 predicts cardiovascular events in patients with hypertension and left ventricular
27
28 hypertrophy: a LIFE study. *J Hypertens* 2004;22:1597-604.
29
30
31

32
33 7. Schirmer H, Omland T. Circulating N-terminal pro-atrial natriuretic peptide is an
34
35 independent predictor of left ventricular hypertrophy in the general population. The
36
37 Tromsø Study. *Eur Heart J* 1999;20:755-63.
38
39
40

41
42 8. Wang TJ, Larson MG, Levy D, et al. Impact of obesity on plasma natriuretic peptide
43
44 levels. *Circulation* 2004;109:594-600.
45
46

47
48 9. Koizumi M, Watanabe H, Kaneko Y, et al. Impact of obesity on plasma B-type
49
50 natriuretic peptide levels in Japanese community-based subjects. *Heart Vessels* 2012;
51
52 27:287-294.
53
54

55
56 10. Olsen MH, Hansen TW, Christensen MK, et al. N-terminal pro brain natriuretic
57
58
59
60

1
2
3
4
5
6 peptide is inversely related to metabolic cardiovascular risk factors and the metabolic
7
8
9 syndrome. *Hypertension* 2005, 46:660-6.

10
11 11. Hamilton MT, Hamilton DG, Zderic TW. Role of low energy expenditure and sitting
12
13 in obesity, metabolic syndrome, type 2 diabetes, and cardiovascular disease. *Diabetes*
14
15 2007;56:2655-67.

16
17
18 12. Avery L, Flynn D, van Wersch A, Sniehotta FF, Trenell MI. Changing physical
19
20 activity behavior in type 2 diabetes: a systematic review and meta-analysis of behavioral
21
22 interventions. *Diabetes Care* 2012;35:2681-89.

23
24
25 13. Barletta G, Stefani L, Del Bene R, et al. Effects of exercise on natriuretic peptides
26
27 and cardiac function in man. *Int J Cardiol* 1998;65:217-25.

28
29
30 14. Nielsen HB, De Palo EF, Meneghetti M, Madsen PL, Ihlemann N, Secher NH.
31
32 Circulating immunoreactive proANP1-30 and proANP31-67 responses to acute exercise.
33
34 *Regul Pept* 2001;99:203-7.

35
36
37 15. American Diabetes Association. Diagnosis and classification of diabetes mellitus.
38
39
40
41
42 *Diabetes Care* 2010;33:S62-9.

43
44
45 16. Levine JA. Non-exercise activity thermogenesis (NEAT). *Nutr Rev* 2004;62:S82-97.

46
47
48 17. Oshima Y, Kawaguchi K, Tanaka S, et al. Classifying household and locomotive
49
50 activities using a triaxial accelerometer. *Gait Posture* 2010;31:370-4.

- 1
2
3
4
5
6 18. Ohkawara K, Oshima Y, Hikihara Y, Ishikawa-Takata K, Tabata I, Tanaka S.
7
8
9 Real-time estimation of daily physical activity intensity by a triaxial accelerometer and
10
11 a gravity-removal classification algorithm. *Br J Nutr* 2011;105:1681-91.
12
13
14 19. Ganpule AA, Tanaka S, Ishikawa-Takata K, Tabata I. Interindividual variability in
15
16 sleeping metabolic rate in Japanese subjects. *Eur J Clin Nutr* 2007;61:1256-61.
17
18
19 20. Energy and protein requirements. Report of a joint FAO/WHO/UNU Expert
20
21 Consultation. *World Health Organ Tech Rep Ser* 1985;724:1-206.
22
23
24 21. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC.
25
26 Homeostasis model assessment: insulin resistance and beta-cell function from fasting
27
28 plasma glucose and insulin concentrations in man. *Diabetologia* 1985;28:412-419.
29
30
31 22. Mongia SK, La'ulu SL, Apple FS, Ler R, Murakami MM, Roberts WL. Performance
32
33 characteristics of the Architect brain natriuretic peptide (BNP) assay: a two site study.
34
35
36
37
38
39
40
41
42
43
44 23. Neeland IJ, Winders BR, Ayers CR, et al. Higher natriuretic peptide levels associate
45
46 with a favorable adipose tissue distribution profile. *J Am Coll Cardiol* 2013;62:752-60.
47
48
49 24. Chainani-Wu N, Weidner G, Purnell DM, et al. Relation of B-type natriuretic
50
51 peptide levels to body mass index after comprehensive lifestyle changes. *Am J Cardiol*
52
53
54
55
56
57
58
59
60

- 1
2
3
4
5
6 25. Mehra MR, Uber PA, Park MH, et al. Obesity and suppressed B-type natriuretic
7
8 peptide levels in heart failure. *J Am Coll Cardiol* 2004;43:1590-5.
9
10
11 26. McCord J, Mundy BJ, Hudson MP, et al. Relationship between obesity and B-type
12
13 natriuretic peptide levels. *Arch Intern Med* 2004;164:2247-52.
14
15
16 27. Dessì-Fulgheri P, Sarzani R, Tamburrini P, et al. Plasma atrial natriuretic peptide and
17
18 natriuretic peptide receptor gene expression in adipose tissue of normotensive and
19
20 hypertensive obese patients. *J Hypertens* 1997;15:1695-9.
21
22
23 28. Das SR, Drazner MH, Dries DL, et al. Impact of body mass and body composition
24
25 on circulating levels of natriuretic peptides: results from the Dallas Heart Study.
26
27
28
29
30
31
32
33
34
35 29. Costello-Boerrigter LC, Burnett JC Jr. A new role for the natriuretic peptides:
36
37 metabolic regulators of the adipocyte. *J Am Coll Cardiol* 2009;53:2078-9.
38
39
40 30. Sengenès C, Berlan M, De Glisezinski I, Lafontan M, Galitzky J. Natriuretic
41
42 peptides: a new lipolytic pathway in human adipocytes. *FASEB J* 2000;14:1345-51.
43
44
45 31. Miyashita K, Itoh H, Tsujimoto H, et al. Natriuretic
46
47 peptides/cGMP/cGMP-dependent protein kinase cascades promote muscle
48
49 mitochondrial biogenesis and prevent obesity. *Diabetes* 2009;58:2880-92.
50
51
52 32. Tsukamoto O, Fujita M, Kato M, et al. Natriuretic peptides enhance the production
53
54
55
56
57
58
59
60

1
2
3
4
5
6 of adiponectin in human adipocytes and in patients with chronic heart failure. *J Am Coll*
7
8
9 *Cardiol* 2009;53:2070-7.

10
11 33. Rajagopalan S, Kurz S, Münzel T, et al. Angiotensin II-mediated hypertension in the
12
13 rat increases vascular superoxide production via membrane NADH/NADPH oxidase
14
15 activation. Contribution to alterations of vasomotor tone. *J Clin Invest*
16
17
18 1996;97:1916-23.
19
20
21

22
23 34. Moro C, Klimcakova E, Lolmède K, et al. Atrial natriuretic peptide inhibits the
24
25 production of adipokines and cytokines linked to inflammation and insulin resistance in
26
27
28 human subcutaneous adipose tissue. *Diabetologia* 2007;50:1038-47.
29
30
31

32 35. Bordicchia M, Liu D, Amri EZ, et al. Cardiac natriuretic peptides act via p38 MAPK
33
34 to induce the brown fat thermogenic program in mouse and human adipocytes. *J Clin*
35
36
37 *Invest* 2012;122:1022-36.
38
39

40 36. Engeli S, Birkenfeld AL, Badin PM, et al. Natriuretic peptides enhance the oxidative
41
42 capacity of human skeletal muscle. *J Clin Invest* 2012;122:4675-9.
43
44

45 37. Polak J, Kotrc M, Wedellova Z, et al. Lipolytic effects of B-type natriuretic peptide
46
47 1-32 in adipose tissue of heart failure patients compared with healthy controls. *J Am*
48
49
50
51
52 *Coll Cardiol* 2011;58:1119-25.
53
54

55 38. Tekes S, Cikim AS. The association of brain natriuretic peptide and insulin
56
57
58

1
2
3
4
5
6 resistance in obesity-related hypertension. *J Hum Hypertens* 2007;21:546-50.
7

8
9 39. Plasqui G, Bonomi AG, Westerterp KR. Daily physical activity assessment with
10
11 accelerometers: new insights and validation studies. *Obes Rev* 2013;14:451-62.
12

13
14 40. Midorikawa T, Tanaka S, Kaneko K, et al. Evaluation of low-intensity physical
15
16 activity by triaxial accelerometry. *Obesity* 2007;15:3031-8.
17

18
19 41. Ekelund U, Sjöström M, Yngve A, et al. Physical activity assessed by activity
20
21 monitor and doubly labeled water in children. *Med Sci Sports Exerc* 2001;33:275-81.
22

23
24 42. Leenders NY, Sherman WM, Nagaraja HN. Energy expenditure estimated by
25
26 accelerometry and doubly labeled water: do they agree? *Med Sci Sports Exerc*
27
28
29
30
31
32 2006;38:2165-72.
33

(figure legends)

Figure 1 Correlation of physical activity level and plasma BNP levels in 60 subjects.

r indicates correlation coefficient analyzed by the Spearman's rank correlation coefficient.

Figure 2 Serum insulin levels (A) and homeostatic model assessment-estimated insulin resistance (HOMA-IR) (B) in high-BNP group (n = 30) and low-BNP group (n = 30).

Data are mean values±SD. We divided subjects into high-BNP group (≥ 8.0 pg/ml) and low-BNP group (< 8.0 pg/ml) by the median values of BNP. Differences in serum insulin levels and HOMA-IR between high-BNP group and low-BNP group were analyzed by the Mann-Whitney U test.

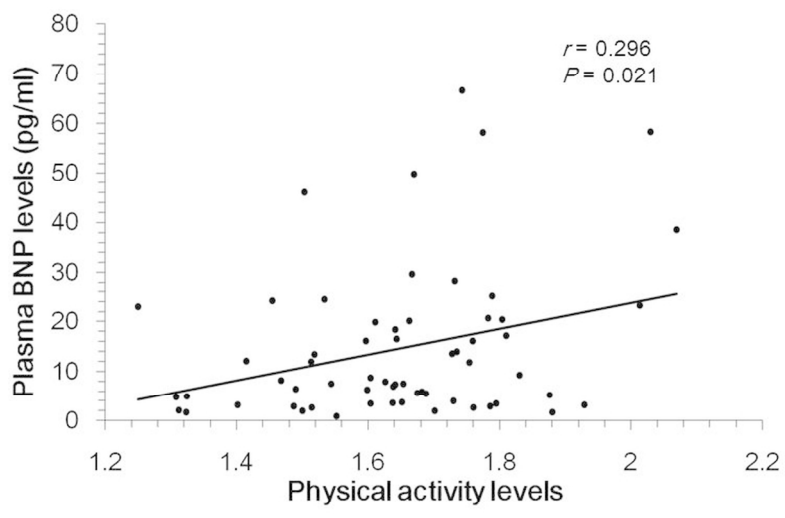
Figure 3 Serum insulin levels (A) and homeostatic model assessment-estimated insulin resistance (HOMA-IR) (B) in each plasma BNP quartiles. Q1 is the lowest quartile and

Q4 is the highest quartile. Boxes indicate medians and quartiles; whiskers indicate the lowest and highest values. We found a significant influence of plasma BNP on both serum insulin (p=0.018 by the Kruskal-Wallis ANOVA) and HOMA-IR (p=0.018). The difference between Q1 and Q2-4 was statistically analyzed by the Scheffe's F Test.

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

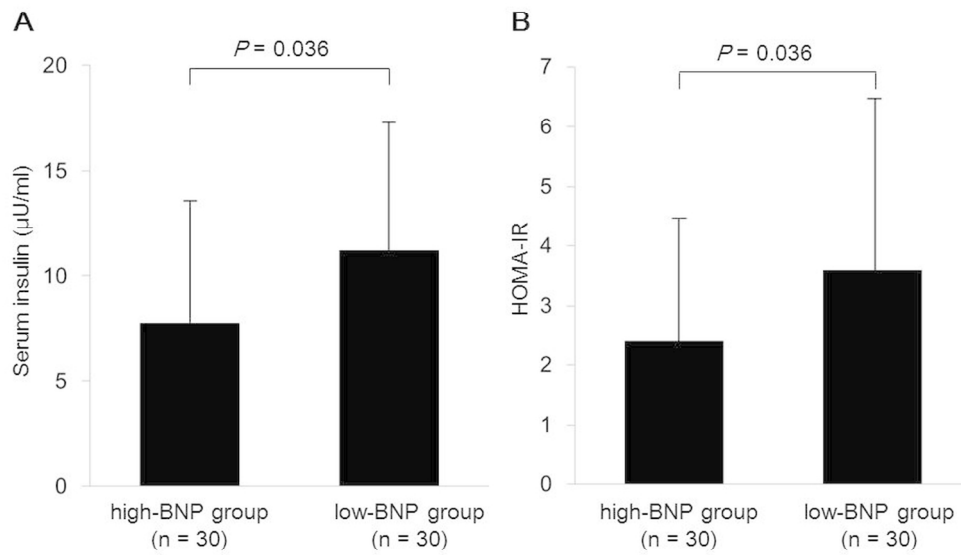
For peer review only

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60



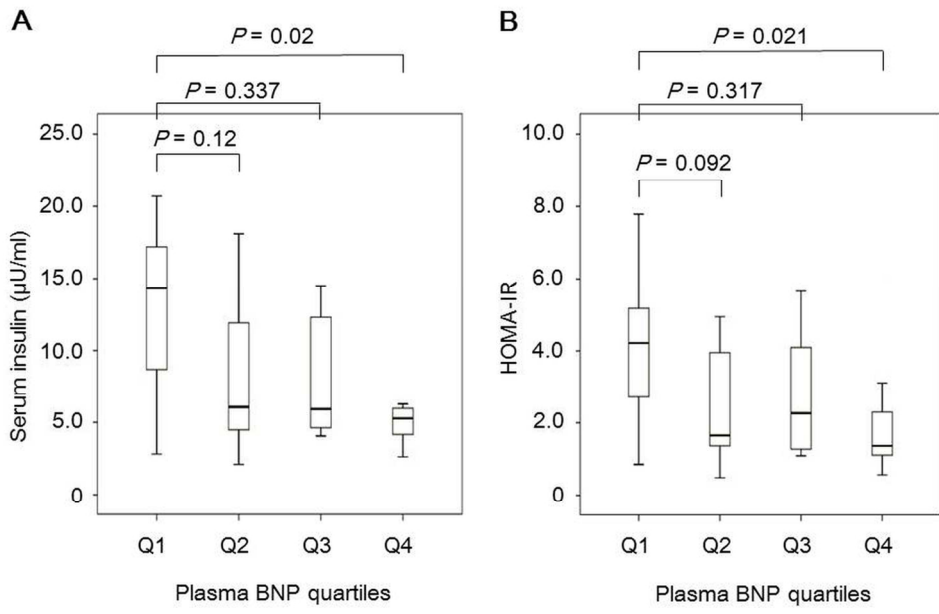
119x90mm (300 x 300 DPI)

ew only



119x90mm (300 x 300 DPI)

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60



119x90mm (300 x 300 DPI)

Review only