PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	The association between daily physical activity and plasma B-type natriuretic peptide in patients with glucose intolerance: a cross-sectional study
AUTHORS	Yanai, Hidekatsu; Hamasaki, Hidetaka; Kakei, Masafumi; Noda, Mitsuhiko; Ezaki, Osamu

VERSION 1 - REVIEW

REVIEWER	Linda Penn Newcastle University, UK
REVIEW RETURNED	04-Sep-2014

GENERAL COMMENTS	I have recommended the need for a statistical opinion. I also suggest that it would be good for someone with specific expertise in the biochemical or physiological mechanisms discussed in this manuscript to review it if at all possible. I am not sure what I can add to the suggestions I have already made in a further review.

REVIEWER	Francesco Tassone Santa Croce e Carle Hospital, Via Michele Coppino Italy
REVIEW RETURNED	14-Oct-2014

GENERAL COMMENTS	The paper is intersting and well written, however I think that some problems in the statistical analysis and in the interpretaion of the results should be addressed by the authorsl. The paper is intersting and well written, however I think that some problems in the statistical analysis and in the interpretaion of the results should be addressed by the authorsl. The paper by Hidetaka Hamasaki et al. is a cross-sectiona study in which the authors investigated the associations of daily physical activity with plasma B-type natriuretic peptide in patients with glucose intolerance. The paper is interesting and well written, however there are, in my opinion, several major statistical problems that could lead to a misinterpretation of the results: - the authors have used a mixture of parametric and non parametric Tests to analyze their data; -BMI, HOMA-IR and insulin levels show often a non-normal distribution: the authors should check for normality; - if the non-normality distribution of these variable would be

	confirmed I think that parametric Parametric Pearson should not be performed (I would suggest Spearman correlation). - If the authors perform a linear multiple regression model they should logarithmically transform the above mentioned variables. - Reanalyzing their data if the authors confirm their previous findings I think that it is not possible to suggest in the discussion (both in the abstract and in the discussion) that BNP levels is beneficially associated with insulin resistance. This I think is the crucial point in my opinion: for example the cardiac ejection fraction is also inversely related with BNP levels but is not beneficial.. BNP levels are reported to increase lipolysis and to deteriorate insulin resistance. I think that the results should be modified.

REVIEWER	Cedric Moro Inserm UMR1048 Institute of Metabolic and Cardiovascular Diseases Obesity Research Laboratory FRANCE
REVIEW RETURNED	18-Nov-2014

GENERAL COMMENTS	This study reports interesting and clinically relevant data. However, although a number of study limitations are acknowledged in the discussion, authors should clarify and address a number of points to improve the manuscript flow and data presentation. Hamasaki et al studied the influence of physical activity level (PAL) on plasma BNP levels measured at baseline in a population of individuals with glucose intolerance and early type 2 diabetes. They report a positive association between PAL and plasma BNP levels independent of age and BMI. They also confirm the negative association between plasma BNP levels and indices of insulin resistance in their population. Overall, the manuscript is clear and concise. However, authors should consider the following points to improve data presentation and manuscript flow. Comments 1. Figure 1 and related results section: authors should at least report the correlations between PAL, age and BMI within the text to justify why they adjusted for these covariates. 2. Figure 2: values for plasma BNP concentrations should be shown for the low- and high-BNP groups. Thus groups were simply made by median. It would be more interesting that authors plot the relationship between quartiles of plasma BNP versus serum insulin and HOMA-IR. 3. Table 2: authors should check if the relationship between plasma BNP and serum insulin and/or HOMA-IR is lost after adjusting for PAL. This would be interesting and novel. 4. Page 12, results: authors should report the details of their regression model with partial β and related p value. they should also indicate if they used a stepwise approach. 5. Authors should also report in the text mean plasma values of BNP and PAL in subjects with IFG, IGT and T2D independently to appreciate the impact of deterioration of blood glucose control on

	systemic BNP levels and PAL. The sex ratio for each group should also be reported. 6. Discussion, page 15: increased plasma BNP levels and/or biological activity could also improve insulin resistance by increasing mitochondrial fat oxidative capacity in white and brown fat, as well as in skeletal muscle as recently shown (Bordicchia et al, J Clin Invest 2012; Engeli et al, J Clin Invest 2012). Minor comments 1. Please provide the inter- and intra-assay CV for the determination of plasma BNP by RIA. 2. Please clarify which plasma BNP was measured, 1-32?

VERSION 1 – AUTHOR RESPONSE

Reviewer 1, Dr. Linda Penn

According to the comment “I have recommended the need for a statistical opinion. I also suggest that it would be good for someone with specific expertise in the biochemical or physiological mechanisms discussed in this manuscript to review it if at all possible. “

We corrected our statistical analyses and discussed biochemical or physiological mechanisms according to Reviewer 2 and 3.

Reviewer 2, Dr. Francesco Tassone
According to the comment “the authors have used a mixture of parametric and non parametric Tests to analyze their data; -BMI, HOMA-IR and insulin levels show often a non-normal distribution: the authors should check for normality;
- if the non-normality distribution of these variable would be confirmed I think that parametric Parametric Pearson should not be performed (I would suggest Spearman correlation).
- If the authors perform a linear multiple regression model they should logarithmically transform the above mentioned variables.

We used Spearman correlation, and made new Table 2 using Spearman correlation.

According to the comment- Reanalyzing their data if the authors confirm their previous findings I think that it is not possible to suggest in the discussion (both in the abstract and in the discussion) that BNP levels is beneficially associated with insulin resistance. This I think is the crucial point in my opinion: for example the cardiac ejection fraction is also inversely related with BNP levels but is not beneficial.. BNP levels are reported to increase lipolysis and to deteriorate insulin resistance. I think that the results should be modified.

We deleted the expression “beneficially”, and also added the following sentences citing new references.
However, the cardiac ejection fraction is also inversely related with plasma BNP levels but is not beneficial,2-7 and plasma BNP levels are reported to increase lipolysis,37 and to be positively associated insulin resistance.38
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.

Reviewer 3, Dr. Cedric Moro
According to the comment “1. Figure 1 and related results section: authors should at least report the correlations between PAL, age and BMI within the text to justify why they adjusted for these covariates.”

We added the following sentences.
Age was correlated with BMI ($r=-0.431$, $p<0.001$), but not correlated with PAL ($r=0.096$, $p=0.462$). BMI was not correlated with PAL ($r=-0.11$, $p=0.4$).

According to the comment “2. Figure 2: values for plasma BNP concentrations should be shown for the low- and high-BNP groups. Thus groups were simply made by median. It would be more interesting that authors plot the relationship between quartiles of plasma BNP versus serum insulin and HOMA-IR.”

We added the following sentences and made new Figure 3.
We also analyzed the relationship between quartiles of plasma BNP versus serum insulin and HOMA-IR (Figure 3). We found a significant influence of plasma BNP on both serum insulin ($p=0.018$ by the Kruskal-Wallis ANOVA) and HOMA-IR ($p=0.018$). Serum insulin level in the highest plasma BNP quartile was significantly lower than in the lowest plasma BNP quartile. HOMA-IR in the highest plasma BNP quartile was also significantly lower than in the lowest plasma BNP quartile.

According to the comment “3. Table 2: authors should check if the relationship between plasma BNP and serum insulin and/or HOMA-IR is lost after adjusting for PAL. This would be interesting and novel.”

We added the following sentences.
Significant correlations of plasma BNP levels with serum insulin ($\beta=-0.204$, $p=0.119$) and HOMA-IR ($\beta=-0.271$, $p=0.129$) were lost after adjusting for PAL.

According to the comment “4. Page 12, results: authors should report the details of their regression model with partial β and related p value. they should also indicate if they used a stepwise approach.”

We added the following sentences.
Age was correlated with BMI ($r=-0.431$, $p<0.001$), but not correlated with PAL ($r=0.096$, $p=0.462$). BMI was not correlated with PAL ($r=-0.11$, $p=0.4$). The values of PAL showed a normal distribution ($p=0.453$ by Shapiro-Wilk test), however, plasma BNP levels showed non-normal distribution ($p<0.001$). Multivariate logistic regression was used after controlling simultaneously for potential confounders. Variables considered in the models were age (continuous) and BMI (continuous). To adjust the correlation between PAL and plasma BNP by age and BMI, plasma BNP values were logarithmically 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 ($\beta=0.282$, $p=0.018$).

According to the comment “5. Authors should also report in the text mean plasma values of BNP and PAL in subjects with IFG, IGT and T2D independently to appreciate the impact of deterioration of blood glucose control on systemic BNP levels and PAL. The sex ratio for each group should also be reported.”

We added the following sentences.
The mean ± SD of plasma BNP levels in patients with type 2 diabetes (13 men and 18 women), IFG (5 men and 9 women) and IGT (10 men and 5 women) were 14.3 ± 13.5 pg/ml, 12.9 ± 17.2 pg/ml and 14.5 ± 15.1 pg/ml, respectively. The mean ± SD of PAL in patients with type 2 diabetes, IFG and IGT

were 1.66 ± 0.22, 1.65 ± 0.15 and 1.66 ± 0.20, respectively. There were no significant differences in plasma BNP levels and PAL among patients with type 2 diabetes, IFG and IGT.

According to the comment “6. Discussion, page 15: increased plasma BNP levels and/or biological activity could also improve insulin resistance by increasing mitochondrial fat oxidative capacity in white and brown fat, as well as in skeletal muscle as recently shown (Bordicchia et al, J Clin Invest 2012; Engeli et al, J Clin Invest 2012).”

We added the following sentences citing two new referenes.
Recently, increased plasma BNP levels and/or biological activity could also improve insulin resistance by increasing mitochondrial fat oxidative capacity in white and brown fat, as well as in skeletal muscle.35 36
35. Bordicchia M, Liu D, Amri EZ, et al. Cardiac natriuretic peptides act via p38 MAPK to induce the brown fat thermogenic program in mouse and human adipocytes. J Clin 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.

Minor comments
According to the comment “1. Please provide the inter- and intra-assay CV for the determination of plasma BNP by RIA.”

We added the following sentences.
Imprecision studies yielded within run CVs of 1.1 to 5.1% and total CVs of 2.3 to 5.3% using human plasma based multi-constituent controls at concentrations of 92, 500, and 3500 ng/L.22

According to the comment “2. Please clarify which plasma BNP was measured, 1-32?

We added the following sentences.
Plasma BNP (BNP 1-32) levels were measured using specific immunoradiometric assay for human BNP (ARCHITECT BNP-JP®, ABBOTT JAPAN Co., Ltd, Tokyo, Japan).22

VERSION 2 – REVIEW

REVIEWER	Francesco Tassone Santa Croce e Carle Hospital, Cuneo, Italy
REVIEW RETURNED	11-Dec-2014

GENERAL COMMENTS	The authors have modified the paper according to the criticisms of the referees.

REVIEWER	Cedric Moro Inserm, Toulouse, France
REVIEW RETURNED	09-Dec-2014

GENERAL COMMENTS	My comments have been properly adressed. I have no further comment.

VERSION 2 – AUTHOR RESPONSE

According to the comment “1. Please amend the title to "The association between daily physical activity and plasma B-type natriuretic peptide in patients with glucose intolerance: a cross-sectional study"

We changed the title of our MS
From
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
To
The association between daily physical activity and plasma B-type natriuretic peptide in patients with glucose intolerance: a cross-sectional study

According to the comment “2. Please include in the methods section how you recruited the participants and why you recruited that number of participants"

We added sentences and rewrote the methods section as the followings.

The study protocol was approved by the Medical Ethics Committee of National Center for Global Health and Medicine (reference number NCGM-G-001212). We recruited study participants with glucose intolerance who did not take any hypoglycemic agents or cholesterol-lowering agents from outpatients who visited the Department of Internal Medicine, National Center for Global Health and Medicine Kohnodai Hospital, between August 2012 and December 2013 (inclusion criteria). Briefly, we determined the existence of glucose intolerance in our outpatients and also diagnosed participants as having type 2 diabetes, IFG and IGT, by performing the 75 g oral glucose tolerance test (OGTT) and the measurement of hemoglobin A1c (HbA1c), fasting plasma glucose (FPG), casual plasma glucose and 2-h values in the OGTT. The participants were diagnosed as having type 2 diabetes according to diagnostic criteria set to serum levels of HbA1c ≧ 6.5 %, FPG ≧ 126 mg/dl, casual plasma glucose ≧ 200 mg/dl and 2-h values in the OGTT ≧ 200 mg/dl.15 The participants were defined as having IFG and IGT according to FPG levels 110 mg/dl to 125 mg/dl, or 2-h values in the OGTT of 140 mg/dl to 199 mg/dl.15 To understand the effects of daily physical activity such as non-exercise activity thermogenesis (NEAT)16 on BNP and metabolic parameters, participants who were engaged in sports-like exercise and resistance training were excluded (exclusion criteria). Participants with heart failure and renal impairment were also excluded (exclusion criteria).

We also added the following sentence in the Result section.

We recruited 60 participants (28 men and 32 women) by the inclusion and exclusion criteria for participation in this study.