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*Original Article*

# The rise of circulatory endothelin (ET)-1 and endothelin receptors ($ET_A$, $ET_B$) expression in kidney of obese wistar rat

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**Abstract:** Background: Endothelin (ET)-1, a circulatory protein, and its receptors ($ET_A$ and $ET_B$) in various organs were reported to play a pivotal role in many diseases, including obesity. However, the changes of $ET_A$ and $ET_B$ expression in ventricle and kidney in obesity was less reported. The study is designed to observe the level of circulatory ET-1 and expression of $ET_A$/$ET_B$ in ventricle and kidney of obese, as compared to non-obese, *Wistar* rats. Methods: Groups of obese 14 and 34 weeks *Wistar* rats were compared to non-obese controls at similar ages. The obesity status was achieved by feeding the with high calories protein diet CP 551 + milk powder, while the control group was fed with a standard calorie protein AD II diet. The concentration of circulatory ET-1, $ET_A$ and $ET_B$ of ventricle and kidney were measured by Enzyme Linked Immunosorbent Assay (ELISA) technique after the termination of both groups at $14^{th}$ and $24^{th}$ weeks. Results: The level of circulatory ET-1, expression of $ET_A$ and $ET_B$ in kidney, and LDL of obese rats were significantly higher than control rats (T-Test, $P<0.05$) in the elder groups, while no differences of the $ET_A$ and $ET_B$ were found in the ventricle. No differences of the levels of circulatory ET-1, $ET_A$ and $ET_B$ expression were found between obese and control groups of younger rats ($P>0.05$). HDL levels were under normal value for both groups. Conclusion: Obesity in elder obese rats leads to dysregulation of kidney vessels through activity of ET-1 and $ET_A$/$ET_B$

**Keywords:** Circulatory ET-1, $ET_A$, $ET_B$, obesity

## Introduction

Endothelin-1 (ET-1) is a chemical substance with 21 amino acids which is produced by endothelial cells [1] and other cells that regulate the caliber of vessels. Endothelin receptor A ($ET_A$) is a G-protein couple receptor with 442 amino acids and has 64% homolog with endothelin receptor B ($ET_B$) which has 442 amino acids [2]. In physiologic condition, the binding of ET-1 to $ET_A$ receptors will stimulate constriction of the vessels, while binding to $ET_B$ receptors dilates the vessels [3, 4]. The $ET_B$ was reported to be up regulated in hypertension to compensate the decreased blood flow, while in other pathologic conditions the increase of $ET_A$ occurs simultaneously with the decrease of $ET_B$, which can cause severe vasoconstriction [4, 5].

Obesity condition could produce cardiovascular diseases through endothelial dysfunction. Vascular dysregulation in ventricle and kidney due to obesity is still unclear [6, 7]. The serum level of ET-1 rises in obesity condition while the $ET_B$ expression decreases in endothelial cell [4, 6]. However there were no many reports on the changes of $ET_A$ and $ET_B$ expression in ventricle and kidney in the obesity.

This study aims to determine the level of circulatory ET-1 and the expression of $ET_A$/$ET_B$ in ventricle and kidney of obese *Wistar* rats. The alteration of $ET_A$/$ET_B$ expression in ventricle and kidney could be an early marker of any dysregulation of vessels in both organs which may develop as cardiovascular diseases in the future.

## Methods

### *Diet*

Both groups, after weaned from the mothers, were given standard calories protein diet AD II chow. To achieve the obesity status, the obese

group was later fed with high calories protein diet CP 551 chow + milk start from the 3rd week (younger group) and 12th weeks (elder group), while the control groups maintained the standard diet. Both the standard and obesity diet were bought from commercial markets. The ingredients of the AD II were of 15% protein and 3% fat, while the CP 551 consists of 18.5-20.5% protein and 4.0% fat. The supplement milk for the obese group consists of 8% protein, 9% fat and 5% carbohydrate. The amount of diet given to control groups were 15 g/day of AD II, the dose was increased to 20 g/day after the 20th week to adjust with the increases of body weight of the rats. The obese groups were initially fed with the same diet as the control groups. At the 3rd week, instead of AD II, the younger obese group was fed with 15 g/day of CP 551 + 7 g/day of milk powder, while the elder obese group was fed with high calories protein diet at week 12th. The dose of CP 551 diet was increased from week 20th for the elder obese group to adjust with body weight increase as for the control group. The obesity condition was reached at 4-6 weeks after feeding with CP 551 and milk, and the obesity status was maintained for about 8-10 weeks before animals were terminated.

Both the control and obese groups were terminated at week 14th and 34th, for the younger and elder aged groups respectively.

*Animals*

Animal used for the experiments are *Wistar* rats cultivated at Animal Houses of University Hasanuddin at Faculty of Medicine. All experimental rats were males. All rats were caged with the standard protocol of animal laboratory of Hasanuddin University with room temperature at 28-30°C. The new born rats were fed by the mothers until two weeks. After two weeks, they were separated from the mothers and divided into 4 groups: 2 for younger groups and 2 for elder groups. Both the younger and elder aged groups were further divided into two groups: obese and control groups. Each group contained five male rats. All the control groups were given standard diet, while the obese group were given the obesity diet as explained above. Obesity of the rats was defined by Rohrer Index = {[bodyweight (gr)]/[naso-anal length (cm)$^3$] × 10$^3$} reached >30. It was categorized as obese if RI >30 [8].

*Laboratory assays*

The rats were terminated by inhaling gas of ether. The blood was drawn from the heart and analyzed using an ELISA Kit (Qiagen) for circulatory ET-1 level. Left ventricle of heart and medulla from the kidney of individual rats were disrupted and macerated by a tissue grinder, for analyses of $ET_A$ and $ET_B$ receptors expressions, using the dedicated ELISA Kits (Qiagen) for the receptors. According to the previous study, $ET_A$ and $ET_B$ are well presented in ventricle of heart and medulla of kidney [9]. The ELISA procedures followed manufacturer's manual.

Lipid profiles such as total cholesterol, LDL and HDL were measured with enzymatic photometric technique (ABX Pentra). The colorimetric indicator is quinoneimine which is generated from 4-aminoantipyrine and phenol by hydrogen peroxide under the catalytic action of peroxidase (Trinder's reaction).

*Statistical test*

Data was analyzed by statistical software SPSS ver. 17.0 and presented in tables by means ± standard deviations. The mean differences of circulatory ET-1 level, $ET_A$/$ET_B$ expressions, and lipid profiles between obese and control groups in younger and elder rats were examined by *t test.*

All procedures for this study were approved by the Institutional Review Board (IRB) for Health and Medical Research of The Faculty of Medicine Universitas Hasanuddin.

## Result

The obesity status of each groups are presented in **Table 1**. It shows significant differences between the obese and the control groups in both younger and elder ages after being fed the obesity diet, CP 551 + milk powder.

**Table 2** shows that the level of circulatory ET-1, $ET_A$ and $ET_B$ expression in kidney of elder obese rats differs significantly compared to the control rats of the same age group, while expression of $ET_A$ and $ET_B$ in ventricle shows no statistical differences. In younger age rats, no statistical differences were found between obese and control groups for all the parameters in

**Table 1.** Weight, length and Rohrer Index of obese and control rats before terminated

| | 14 weeks old Wistar rat | | | 34 weeks old Wistar rat | | |
|---|---|---|---|---|---|---|
| | Obese | Control | P* | Obese | Control | P* |
| Weight (gr) | 441,0 ± 25,6 | 213,8 ± 40,3 | | 361,2 ± 22,8 | 248,4 ± 41,5 | |
| Length (cm) | 21,8 ± 0,5 | 20,1 ± 0,7 | | 21,8 ± 0,8 | 20,6 ± 0,9 | |
| Rohrer Index | 42,6 ± 2,2 | 26,1 ± 2,4 | <0.001 | 35,1 ± 4,6 | 28,1 ± 1,6 | 0.013 |

*Calculated with an independent sample t test.

**Table 2.** Level of circulatory ET-1, and ETA/ETB expression in ventricle and kidney

| | 14 weeks old Wistar rat | | | 34 weeks old Wistar rat | | |
|---|---|---|---|---|---|---|
| | Obese | Control | P* | Obese | Control | P* |
| ET-1 (pg/ml) | 19,500 ± 9,561 | 16,048 ± 5,207 | 0,498 | 23,048 ± 2,017 | 14,710 ± 2,358 | 0,001 |
| ETAh (pg/ml) | 3,138 ± 0,953 | 2,187 ± 0,924 | 0,174 | 2,628 ± 0,934 | 1,862 ± 0,763 | 0,217 |
| ETAk (pg/ml) | 1,250 ± 0,722 | 1,327 ± 0,229 | 0,826 | 1,790 ± 1,008 | 0,531 ± 0,262 | 0,030 |
| ETBh (pg/ml) | 2,262 ± 0,325 | 2,314 ± 0,218 | 0,774 | 2,160 ± 0,193 | 1,794 ± 0,331 | 0,065 |
| ETBk (pg/ml) | 3,148 ± 0,217 | 3,128 ± 0,184 | 0,879 | 2,960 ± 0,329 | 1,826 ± 0,391 | 0,001 |

*Calculated with an independent sample t test. ETAh = endothelin receptor A in ventricle, ETAk = endothelin receptor A in kidney. ETBh = endothelin receptor B in ventricle, ETBk = endothelin receptor B in kidney.

**Table 3.** Lipid profile of obese wistar rat and control

| | 14 weeks old Wistar rat | | | 34 weeks old Wistar rat | | |
|---|---|---|---|---|---|---|
| | Obese | Control | P* | Obese | Control | P* |
| Chol total (mg/dl) | 59,750 ± 6,751 | 29,250 ± 11,899 | 0,004 | 59,800 ± 5,019 | 45,600 ± 5,319 | 0,002 |
| HDL (mg/dl) | 26,800 ± 3,347 | 10,600 ± 5,272 | 0,000 | 22,400 ± 3,578 | 15,400 ± 2,881 | 0,009 |
| LDL (mg/dl) | 20,000 ± 5,244 | 27,200 ± 15,023 | 0,341 | 60,000 ± 18,403 | 30,200 ± 11,756 | 0,021 |

*Calculated with an independent sample t test. Chol total = Cholesterol total, HDL = high density lipoprotein, LDL = low density lipoprotein.

both heart and kidney, as well as in circulatory parameters.

Lipid profiles of total cholesterol, HDL and LDL in serum are significantly higher in the obese group compared to the control group of elder rats, but only total cholesterol and HDL of obese group of the younger rats are higher than the non-obese rats (**Table 3**).

## Discussion

The present study revealed that established obesity in elder age rats increases the level of circulatory ET-1 and expression of $ET_A/ET_B$ in kidney. The data support the current believes that obesity contributes to dysregulation in kidney vessels through endothelin-1 system and its receptor. Increase of ET-1 and $ET_B$ receptors in kidney could be mediated by up regulation process to overcome the problem in kidney blood flow, since the binding of the ET-1 to $ET_B$ receptors stimulates vasodilatation of blood vessels. This is in line with results of few studies on circulatory ET-1 and of $ET_A/ET_B$ expression in kidney of obesity condition [6, 11, 12]. Another function of ET-1 and $ET_A/ET_B$ in kidney is to inhibit Na reabsorption to regulate normal blood pressure [13] that may increase in this study. Increase of $ET_A$ and $ET_B$ expression in our data could be the compensation mechanism to normalize the blood pressure.

In heart tissue, expression of $ET_A$ and $ET_B$ receptors in obese rats of elder age also showed a tendency of increasing if compared to the control group, yet statistically not significant. Blood flow to ventricle walls could decrease but well compensated by up regulation of $ET_B$, and this change could be the minimal alteration of vessels. In heart failure patients, it was reported that serum ET-1 and $ET_A/ET_B$ were increased above normal level [5, 14]. A study with use of dual antagonist of ET-1 receptors revealed that

ejection fraction of heart increased by inhibition of both $ET_A/ET_B$ receptors [14]. The increase of $ET_A$ leads to vasoconstriction and precipitates heart failure and hypertension, while increase of $ET_B$ was needed to clear the ET-1 in circulation to prevent the severity of diseases [14, 16, 17]. However, the effects of obesity in ventricle's vessels, as well as in kidney, in this study were not directly measured.

Our data suggest that obesity could be related to dysregulation of blood vessels and it is more prominent in medulla of kidney compared to ventricle's vessels. The increase of $ET_A/ET_B$ expression was a marker for reducing blood flow that may harm the kidney tissue. Although this finding should be carefully interpreted but this study revealed that dysregulation of kidney vessels in obesity could be an early sign of a developing pathologic condition of the kidney and lead to apparent kidney diseases.

Age is an independent factor for ET-1 and its receptors activity as shown in our data. The significant increase of circulatory ET-1 level and $ET_A/ET_B$ expressions was found in obese group of elder age rats, but not in younger one (**Table 2**). It appeared that obesity in elder rat caused vessels dysfunction of kidney and did not occur in younger rat. This data was supported by studies that aging in rats and human will increase the activity of ET-1 and its receptor in kidney [7, 18]. Our data showed that obesity has different impact in different age groups, and it can produce the significant alteration in elder rat. Although in this study, elder and younger rats could be in the same phase of aging but it seemed that diet induced obesity has a significant impact to the level of circulatory ET-1 and its receptor.

Most of lipid profiles such as serum level of total cholesterol, HDL and LDL were significantly increased in obese individuals in both younger and elder age rat. In this study, although still in a normal range [10], the level of total cholesterol of the blood of the rats tended to increase in all obese group, while LDL level in obese group increased higher than the control group only in elder age rats (**Table 3**). Cholesterol and LDL have detrimental effect to the vessels if their concentration in the blood is increased [10]. High level of LDL and low level of HDL will influence the vessel's healthy. The hyperlipidemia in obese rats in this study may affect the endothelial cells especially for kidney through ET-1 and $ET_A/ET_B$ stimulation. Another study in animal showed that high fat diet fed rats lead to microvascular damage of vessels and increase of ET-1 and $ET_A/ET_B$ receptors [19]. ET-1 also been reported to induce proliferation of pre adipocytes [20] which could affect the vessels wall. Microangiopathy found in type 2 diabetes has positive correlation to the level of ET-1 [21].

The study shows that although the alteration rate of the physical status such as weight and length is higher in younger rats (**Table 1**), the effects of diet to the physiological conditions are more obvious in elder age rats. These data may have at least two implications: 1) The observed phenomenon might be due to a more flexible homeostasis system in younger rats, hence, better compensation mechanism to maintain the homeostasis status than those of elder rats. So, effect of pathological condition may establish better in elder age rats (**Tables 2**, **3**). This suggest that it is worth analyzing data on elder age individuals to see the effect of pathological changes; 2) The finding that the elder-onset obesity diet increased blood ET-1 levels and selectively increased ET receptor components in the kidney but not heart, while in contrast, only minimal change was observed in younger rats. The role of the ET system in comorbidities of obesity opens a new insight to the understanding of the disease mechanisms. Based on the data, the level of $ET_A$ and $ET_B$ expression should be considered as a marker for endothelial dysfunction in kidney vessels, and an endothelin receptor antagonist may be an option in the treatment of obesity-associated kidney diseases.

We conclude that obesity could make dysregulation of kidney vessels through activity of ET-1 and $ET_A/ET_B$ especially in elder obese rats. Limitations of the present study, e.g. th no pathological examination for dysregulation of vessels in kidney and ventricles, and no direct measurements of blood pressure change, do not eliminate the potential of the findings presented for further studies on the obesity-related damage of vessels in specific organs, or for finding novel therapies in the future.

**Disclosure of conflict of interest**

None.

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