Introduction

The renin-angiotensin-aldosterone system (RAAS) is involved in the regulation of blood pressure and salt/water balance. Increased aldosterone levels are found in states of disturbed energy balance such as the metabolic syndrome. Adipose tissue has been recognized to play a pivotal role in the regulation of energy homeostasis. We investigated direct aldosterone effects on brown adipocyte function. Aldosterone dose-dependently inhibited expression of uncoupling protein-1 (UCP-1) by 30% (p < 0.01). Furthermore, aldosterone dose-dependently impaired insulin-induced glucose uptake by about 25% (p < 0.01). On a transcriptional level, mRNA of the proinflammatory adipokines leptin and monocyte chemoattractant protein-1 (MCP-1) was increased by 5,000% and 40%, respectively, by aldosterone exposure (p < 0.05). This study demonstrates that aldosterone directly impacts on major adipose functions including stimulation of proinflammatory adipokines.

Key words
Adipose tissue · Metabolic syndrome · Monocyte-chemoattractant protein-1

Abstract

Aldosterone is a mineralocorticoid hormone that regulates blood pressure and salt/water balance. Increased aldosterone levels are found in states of disturbed energy balance such as the metabolic syndrome. Adipose tissue has been recognized to play a pivotal role in the regulation of energy homeostasis. We investigated direct aldosterone effects on brown adipocyte function. Aldosterone dose-dependently inhibited expression of uncoupling protein-1 (UCP-1) by 30% (p < 0.01). Furthermore, aldosterone dose-dependently impaired insulin-induced glucose uptake by about 25% (p < 0.01). On a transcriptional level, mRNA of the proinflammatory adipokines leptin and monocyte chemoattractant protein-1 (MCP-1) was increased by 5,000% and 40%, respectively, by aldosterone exposure (p < 0.05). This study demonstrates that aldosterone directly impacts on major adipose functions including stimulation of proinflammatory adipokines.

Key words
Adipose tissue · Metabolic syndrome · Monocyte-chemoattractant protein-1

Affiliation
1 Department of Internal Medicine I, University of Lübeck, Germany
2 Department of Internal Medicine III, University of Leipzig, Germany

Remark
* Both authors contributed equally to this work.

Correspondence
J. Klein, M.D. · Department of Internal Medicine I, University of Lübeck · Ratzeburger Allee 160 · 23538 Lübeck · Germany · Fax: +49 451 500 4193 · E-Mail: j.klein@uni-luebeck.de

Received: 17 March 2005 · Accepted after revision: 28 April 2005

Bibliography
Accumulating evidence suggests a link between mineralocorticoid actions, adipose tissue, and the pathogenesis of features of the metabolic syndrome [10,11]. Adipose tissue may contribute to the systemic RAAS by expression and secretion of angiotensinogen and other components of a local RAAS [12]. Aldosterone itself does not appear to be synthesized in fat cells. However, expression of the mineralocorticoid receptor can be found in adipose tissue [13]. Here, we investigate molecular and functional effects of aldosterone on adipocyte metabolism and endocrine activity. Our study demonstrates a direct impact of this mineralocorticoid hormone on thermogenesis, insulin sensitivity, and regulation of anorexigenic and proinflammatory adipokines.

Materials and Methods

Materials
Aldosterone was purchased from Sigma-Aldrich Co. (St. Louis, MO, USA) and Merck Bioscience (Darmstadt, Germany). Phosphospecific antibodies against p44/p42 mitogen-activated protein (MAP) kinase, and Akt (S473) were from Cell Signaling Technology Inc. (Beverly, MA, USA). Glucose uptake assays were performed with 2-deoxy-[3H]glucose from NEN Life Technologies (Deirich, Germany). Primers for expression analysis were ordered from BIOMER (Göttingen, Germany) and TIB Molbiol (Berlin, Germany). All other chemicals were from Sigma-Aldrich Co. (St. Louis, MO, USA) unless stated otherwise.

Cell Culture
We used SV40 T-immortalized murine brown preadipocytes in all experiments [14,15]. Cells were cultured in differentiation medium consisting of Dulbecco’s modified Eagle’s medium (DMEM; Invitrogen, Paisley, Scotland) with 4.5 g/l glucose and supplements of 20 nmol/l insulin, 1 nmol/l triiodothyronine, 20 % fetal bovine serum, and penicillin/streptomycin (Deirich, Germany). All other chemicals were from Sigma-Aldrich Co. (St. Louis, MO, USA) and Merck Bioscience (Darmstadt, Germany). Phos-phate-buffered saline (0.15 mol/l NaCl, 5.4 mmol/l KCl, 1 mmol/l MgCl2, 1 mmol/l CaCl2, pH 7.4) and RPMI-1640 medium (Gibco, Paisley, Scotland) were used. Left-overs from DMEM were added for seven days. Cells were starved overnight either with rinsing buffer (10 mmol/l Tris, 150 mmol/l NaCl, 0.05 % Tween 20, pH 7.2) supplemented with 3 % bovine serum albumin (‘blocking solution’). Membranes were then incubated with the antibodies for 1–2 h. Protein bands were visualized using chemiluminescence (Roche Molecular Biochemicals, Mannheim, Germany) with enhanced chemiluminescence films (Amersham Pharmacia Biotech, Freiburg, Germany).

Analysis of gene expression
UCP-1, leptin, and MCP-1 mRNA expression was determined by reverse transcription (RT) and real-time polymerase chain reaction (PCR). Total RNA was isolated using RNAwitz (Ambion, Austin, TX, USA) or TRIzol reagents (Invitrogen, Karlsruhe, Germany), followed by RNA clean-up using the RNasey kit (Qiagen, Hilden, Germany). RNA quality was tested by photometric analysis. RNA analysis, the following primers were used: UCP-1 (acc. nos. M21222 and M21244) 5'-ATG GTG ACG ACC ACA ACT TCC GAA GTG-3' (sense) and 5'-GTA CCT GAA GCC TGG TTG TCA TTG-3' (antisense); leptin (acc. no. NM008493) 5'-GTT CCT ATC CAG AAA GTC CAG GAT G-3' (sense) and 5'-CTG GTG AGG ACC TGT TGA TAG AC-3' (antisense); MCP-1 (acc. no. NM011333) 5'-GCC CCA CTC ACC TGC TGC TAC T-3' (sense) and 5'-CTT GCT GGT GAT CCT CTT GT-3' (antisense). PCR was performed as follows: initial denaturation at 95 °C for 90 s, 40 cycles with 95 °C for 30 s, 60 °C for 60 s, 72 °C for 60 s (MCP-1) or 95 °C for 30 s, 56 °C for 30 s, 72 °C for 30 s (UCP-1, leptin). Specific amplification was confirmed using melting curve profiles. Optimized relative quantification was done using the Relative Expression Software Tool (REST®) [16] normalized to 36B4 as housekeeping gene [17].

Glucose uptake assay
Glucose uptake assays were essentially carried out as described elsewhere [14]. In brief, fully differentiated cells were starved overnight in serum-free medium and then washed twice in Krebs-Ringer HEPES. Cells were then stimulated with or without insulin (100 nM) for 30 min. At the end of the stimulation period, the cells were incubated with 2-deoxy-[3H]glucose for 4 min, washed in phosphate-buffered saline, and lysed with 0.1 % sodium dodecyl sulfate (SDS). The incorporated radioactivity was determined by liquid scintillation counting.

Western blotting
Fully differentiated cells were starved for up to 24 h in serum-free-medium prior to stimulation. Cells were washed with ice-cold phosphate-buffered saline and proteins were isolated using whole-cell lysis buffer containing 10 μg/ml aprotinin, 10 μg/ml leupeptin, 2 mmol/l vanadate and 26 mmol/l phenylmethylsulfonyl fluoride (Fluka Chemie, Neu-Ulm, Germany). Protein content was measured by the Bradford method using the dye from Bio-Rad (Hercules, CA, USA). Whole-cell protein lysates were subjected to SDS polyacrylamide gel electrophoresis. Proteins were then transferred to nitrocellulose membranes. Membranes were blocked overnight either with rinsing buffer (10 mmol/l Tris, 150 mmol/l NaCl, 0.05 % Tween 20, pH 7.2) supplemented with 3 % bovine serum albumin (‘blocking solution’). Membranes were then incubated with the antibodies for 1–2 h. Protein bands were visualized using chemiluminescence (Roche Molecular Biochemicals, Mannheim, Germany) with enhanced chemiluminescence films (Amersham Pharmacia Biotech, Freiburg, Germany).

Statistical analysis
Statistical analysis was performed using Sigma Stat (SPSS Science, Chicago, IL, USA). Data are presented as means ± SEM. Statistical significance was determined using the unpaired Student t-test or Mann-Whitney U-test as appropriate. Values of p < 0.05 were considered significant, and p < 0.01 highly significant.

Results
Aldosterone inhibits expression of thermogenic UCP-1
Given a potential role for aldosterone in energy homeostasis [4], we studied the direct effect of aldosterone treatment on the ex-
pression of thermogenic UCP-1 protein. Stimulation of fully differentiated brown adipocytes with 100 nmol/l aldosterone for eight hours diminished basal UCP-1 mRNA by 30% (Fig. 1). This effect was dose-dependent, and independent of any effect of aldosterone on adipocyte differentiation as measured by Oil Red-O staining (data not shown).

Aldosterone induces insulin resistance
UCP-dependent thermogenesis is coupled to glucose uptake. We found that insulin, but not aldosterone, induced basal glucose uptake about ten-fold (Fig. 2a). However, when pre-treating cells with aldosterone for twenty-four hours prior to the stimulation with insulin, glucose uptake was dose-dependently reduced by up to 25% (Fig. 2a). Concomitantly, the activation of the major insulin signaling elements protein kinase B and mitogen-activated protein (MAP) kinase as measured by phosphospecific Western blotting was diminished by aldosterone pre-treatment (Fig. 2b).

Aldosterone stimulates leptin and MCP-1 expression
Leptin is the prototypic anorexigenic adipokine [18], and MCP-1 is a proinflammatory chemokine that has been implicated in the pathogenesis of insulin resistance [19, 20]. MCP-1 mRNA expression transiently increased to 140% of basal levels after stimulation with 100 nmol/l aldosterone for 0.5 to 1 h (Fig. 3a). This effect was non-significantly sustained for up to eight hours (data not shown). Moreover, thirty minutes of aldosterone treatment strongly augmented leptin mRNA expression to 5,000% (Fig. 3b). Again, this effect appeared to be sustained for up to eight hours (data not shown).

Discussion
In the present study, we demonstrate direct aldosterone action in adipocytes resulting in reduced thermogenesis, impaired insulin sensitivity, and altered proinflammatory hormone expression.

Individuals with the metabolic syndrome are prone to have an activated RAAS with elevated aldosterone serum levels [4]. Aldosterone interferes with insulin secretion and/or signaling in these patients [4]. Adipocytes importantly contribute to the control of energy homeostasis. In this study, aldosterone depressed basal UCP-1 mRNA levels. Consistent with our findings, aldosterone has been reported to impair UCP-1 mRNA expression induced by isoproterenol and retinoic acid, with doses and kinetics comparable to our results in a brown adipose tumor cell line [21]. However, this is the first study to demonstrate direct effects of aldosterone on basal UCP-1 levels. Although UCP-1 is a marker of adipocyte differentiation, the effect shown here is not caused by impaired differentiation since adipocyte differentiation was unaltered under chronic direct aldosterone exposure (data not shown). Indeed, aldosterone has been reported to facilitate rather than hinder adipocyte differentiation [22].
Coupled to this impairment of UCP-1 expression, we found a direct inhibition of insulin-induced intracellular glucose uptake. Accordingly, insulin signaling elements such as protein kinase B and mitogen-activated kinase were inhibited.

Finally, we have characterized the modulation of adipose endocrine function by aldosterone. As we [23] and others [24] have demonstrated, brown adipocytes produce and secrete leptin, a peptide hormone with central and peripheral effects on multiple physiologic systems including energy homeostasis and the immune system [25]. In this study, we have described a strong stimulation of leptin mRNA expression in cultured adipocytes. In contrast to our findings, patients suffering from primary hyperaldosteronism appear to have decreased leptin levels [26, 27]. This discrepancy may highlight differences between acute and chronic effects of this mineralocorticoid hormone. Furthermore, aldosterone induces the expression of MCP-1, a proinflammatory adipokine that has recently been implicated in insulin resistance and the metabolic syndrome [20]. Thus, aldosterone action may favor a proinflammatory profile of adipokine secretion that mediates cardiovascular complications associated with the syndrome [28].

In summary, this study describes direct actions of aldosterone on adipocyte metabolism and endocrine function. The mineralocorticoid hormone impairs thermogenesis, induces insulin resistance, and modulates the expression of proinflammatory adipokines. These functional changes may play a pathogenic role in states characterized by an activated RAAS such as the metabolic syndrome.

Acknowledgments

J. K. is a Feodor Lynen Fellow of the Alexander von Humboldt Foundation. The expert help of Dr. Andreas Dalski is gratefully acknowledged. This study was supported by a grant from the Deutsche Forschungsgemeinschaft to J. K. (KL 1131/2-5).

References

1. Isomaa B. A major health hazard: the metabolic syndrome. Life Sci 2003; 73: 2395–2411
21. Viengchareun S, Penfornis P, Zennaro MC, Lombes M. Mineralocorticoid and glucocorticoid receptors inhibit UCP expression and function


26 Torpy DJ, Bornstein SR, Taylor W, Tauchnitz R, Gordon RD. Leptin levels are suppressed in primary aldosteronism. Horm Metab Res 1999; 31: 533–536
