Hindawi Publishing Corporation
Experimental Diabetes Research
Volume 2012, Article ID 628978, 12 pages
doi:10.1155/2012/628978

Review Article

Autophagy as a Therapeutic Target in Diabetic Nephropathy

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Received 14 May 2011; Revised 1 August 2011; Accepted 17 August 2011

Academic Editor: Ki-Up Lee

Diabetic nephropathy is a serious complication of diabetes mellitus, and its prevalence has been increasing worldwide. Therefore, there is an urgent need to identify a new therapeutic target to prevent diabetic nephropathy. Autophagy is a major catabolic pathway involved in degrading and recycling macromolecules and damaged organelles to maintain intracellular homeostasis. The study of autophagy in mammalian systems is advancing rapidly and has revealed that it is involved in the pathogenesis of various metabolic or age-related diseases. The functional role of autophagy in the kidneys is also currently under intense investigation although, until recently, evidence showing the involvement of autophagy in the pathogenesis of diabetic nephropathy has been limited. We provide a systematic review of autophagy and discuss the therapeutic potential of autophagy in diabetic nephropathy to help future investigations in this field.

1. Introduction

The prevalence of diabetes mellitus has been increasing worldwide during recent years, and this is estimated to continue in the future [1, 2]. Diabetic nephropathy is a serious complication of diabetes mellitus and is the most common cause of end-stage renal disease [3, 4]. The increasing prevalence of diabetes mellitus and its complications, including diabetic nephropathy, has therefore become a major health problem worldwide. There is now an urgent need to identify new therapeutic target molecules or cellular processes that underlie the pathogenesis of diabetic nephropathy to establish an additional therapeutic option.

Hyperglycemia-mediated alteration of extra- and intracellular metabolism, such as advanced glycation end products [5], increased protein kinase C activity [6], and abnormal polyol metabolism [7], has been recognized as classical pathogenesis of diabetic nephropathy. In addition, intracellular stress associated with renal hypoxia [8, 9], mitochondrial reactive oxygen species (ROS) [10–13], and endoplasmic reticulum (ER) stress [14–16] has recently been proposed and focused as new pathogenesis of diabetic nephropathy. Thus, to maintain the cellular homeostasis against stress condition derived from organelle dysfunction or hypoxia may be a new therapeutic target of diabetic nephropathy.

Autophagy, a lysosomal protein degradation pathway in cells, plays a crucial role in removing protein aggregates as well as damaged or excess organelles to maintain intracellular homeostasis and cell integrity [17]. It has recently been highlighted because it can be stimulated by multiple types of cellular stressors including starvation, hypoxia, or ER stress. The study of autophagy in mammalian systems and in disease states is advancing rapidly, and many investigators are entering this new and exciting field (Figure 1). It has been revealed that autophagy plays a crucial role in several organs, especially in metabolic organs, and that its alteration is involved in the pathogenesis of metabolic [18–21] and age-related diseases [22–27]. The functional role of autophagy in the kidneys is currently under intense investigation (Figure 1), and it has been revealed that autophagy has a renoprotective role in several animal models including those used for aging and acute kidney injury [26–31]. However, the role of autophagy in diabetic nephropathy remains unclear.

Alteration of several nutrient-sensing pathways is related to the development of metabolic diseases, such as type 2 diabetes and its vascular complications. Major nutrient-sensing

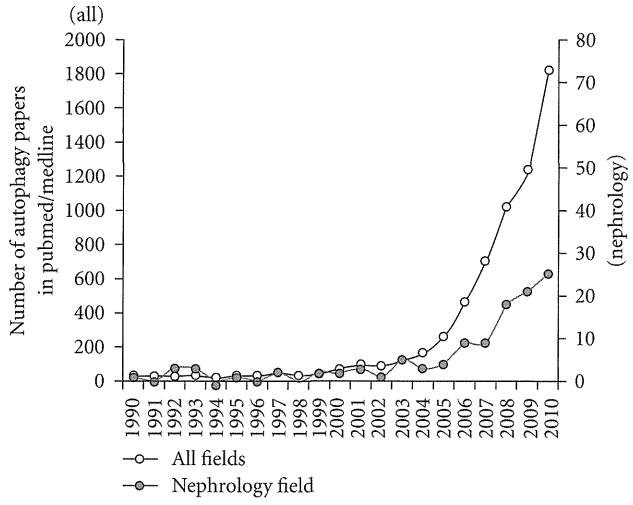

FIGURE 1: *Substantial increases in the number of autophagy-related papers indexed in PubMed/Medline.* The number of autophagy-related papers in all fields has increased remarkably over recent decades. Corresponding with the increase of autophagy-related papers in all fields, publication of autophagy-related papers in nephrology fields, also gradually increased.

pathway involves the mammalian target of rapamycin (mTOR) [32–35], AMP-activated protein kinase (AMPK), [36–40] and oxidized NAD- (NAD^+-) dependent histone deacetylase (Sirt1) [41–43], which are also recognized as the regulatory factors of autophagy under nutrient-depleted condition. As described above, autophagy can be induced by intracellular stresses that are involved in the pathogenesis of diabetic nephropathy [44]. Thus, alteration of these nutrient-sensing pathways under diabetic condition may impair the autophagic stress response stimulated by intracellular stress, which may lead to exacerbation of organelle dysfunction and subsequent diabetic nephropathy.

The above findings lead us to hypothesize that autophagy is involved in the pathogenesis of diabetic nephropathy and is a potential therapeutic option. Therefore, we provide a systematic review of autophagy and discuss its therapeutic potency in diabetic nephropathy to help future investigations in this field.

2. Autophagy

The term autophagy is derived from Greek and means self-eating. It is highly conserved from yeast to mammals and is a bulk degradation process that is involved in the clearance of long-lived proteins and organelles. Autophagy has two major roles in cells: to recycle intracellular energy resources in response to nutrient-depleted conditions and to remove cytotoxic proteins and organelles under stressful conditions. Autophagy works to maintain cell homeostasis under various stressful conditions. Several types of autophagy have been recognized in cells: macroautophagy, microautophagy, and chaperone-mediated autophagy; these differ in their mechanisms and functions [45, 46]. Of these three types of autophagy, macroautophagy is most prevalent and hereafter is referred to as autophagy. In this paper, we focus on the mechanisms and functions of autophagy.

3. Molecular Mechanisms of Autophagy

During macroautophagy, *de novo* isolation membranes (phagophores) elongate and fuse while engulfing a portion of the cytoplasm within double-membraned vesicles (autophagosomes) (Figure 2). Several origins of autophagosomes have been reported, including the ER [47–49], mitochondria [50], and plasma membrane [51]. Four major steps are involved in the formation of autophagosomes: initiation, nucleation, elongation, and closure. During these steps, autophagy-related proteins are involved (Figure 2). Autophagy is initiated by the unc-51-like kinase (Ulk) 1 (mammalian ortholog of the yeast autophagy-related genes (Atg)1) complex, which is composed of Ulk1 Ser/Thr protein kinase, Atg13, and FIP200 (mammalian homolog of the yeast Atg17) [52–54]. The phosphorylation of Atg13 and FIP200 by Ulk1 is essential for triggering autophagy. Phagophore nucleation is dependent on Beclin 1 (Atg6 in yeast)—an hVps34 or class III phosphatidylinositol 3-kinase (PI3K) complex, which consists of hVps34, hVps15, Beclin 1, and Atg14 [55, 56].

During autophagosome elongation/closure, two dependent ubiquitin-like conjugation systems are involved: Atg12 and LC3 (the mammalian ortholog of the yeast Atg8) [57]. The Atg12-Atg5 conjugate, which forms the Atg12-Atg5-Atg16 complex, contributes to the stimulation and localization of the LC3 conjugation reaction. The cytosolic isoform of LC3 (LC3-I) is conjugated to phosphatidylethanolamine through two consecutive ubiquitination-like reactions that are catalyzed by E1-like enzyme Atg7 and the E2-like enzyme Atg3 to form LC3-II [58]. Thus, LC3-II formation is recognized as a marker of existence of autophagosomes in cell or animal experiments [59–61]. After formation, the autophagosomes merge with the lysosomal compartment to form autolysosomes. The protein p62, also known as sequestosome 1 (SQSTM1), is known to localize to autophagosomes via LC3 interaction and to be constantly degraded by the autophagy-lysosome system [62, 63]. The accumulation of p62 is observed in autophagy-deficient cells [62, 63].

4. Methods Available for Monitoring Autophagy

It is necessary to keep in mind several important points as we monitor and assess the autophagy activity to prevent misconceptions. Some reviews about the methods for autophagy research have been published [59–61]. As briefly below summarized, some methods including electron microscopy (EM), detection of endogenous LC3 or green fluorescent protein (GFP)-LC3 by fluorescence microscopy, and detection of LC3-II by Western blotting are useful in monitoring the number of autophagosomes. However, these methods have some limitations. An accumulation of autophagosomes does not always mean increased formation of autophagosomes and may represent inhibited maturation of autolysosomes (or amphisomes). Simply counting

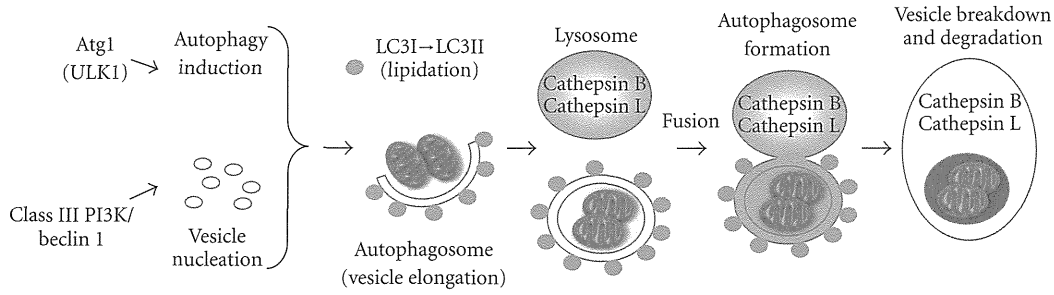

FIGURE 2: *Scheme of autophagic pathways.* Autophagic pathways consist of four steps: initiation, nucleation, elongation, and closure. Autophagy is initiated by the nucleation of an isolation membrane (phagophore). The phagophore elongates and closes on itself to form an autophagosome. Fusion of an autophagosome with a lysosome forms an autolysosome and, where the acid hydrolases in the lysosome, breaks down the inner membrane and cytoplasmic contents.

the number of autophagosomes is insufficient for assessing autophagy activity. Autophagy flux is a term that represents a serial process of autophagy, including the synthesis of autophagosomes, the delivery of cargo to lysosomes, and the degradation of autolysosomes. To distinguish whether the accumulation of autophagosomes is caused by induction of autophagy or inhibition of autophagosome maturation and/or degradation of autophagic substrates in the lysosome, and then assess autophagy activity, an autophagy flux assay is more reliable than counting the number of autophagosomes. There are some useful assays to monitor autophagy flux. These include the LC3 turnover assay, or measurement of total levels of autophagic substrates such as LC3, GFP-LC3, and p62. Furthermore, several types of autophagy inhibitors and activators have recently become available to modulate the activity of autophagy processes. Pharmacological inhibitors of autophagy are PI3-kinase inhibitors such as wortmannin, LY294002, or 3-methladenine (3-MA) and inhibitors that block autophagosome-lysosome fusion or degradation of autophagic cargo in autolysosomes, such as E64d, pepstatin A, and bafilomycin A. However, a major problem is that there are no highly specific inhibitors or activators of autophagy. Thus, it is strongly recommended that pharmacological studies should be combined with studies that investigate deficiency/reduction of autophagy-related genes by genetic knockout/knockdown of ATG genes or dominant-negative mutant autophagy proteins, including Atg3, Atg5, Atg7, and Beclin 1.

5. Role of Nutrient Stress in Autophagy and Diabetic Nephropathy

The kidney is a structurally complex organ and is essential in several functions including excretion of the waste products of metabolism, regulation of body fluid volume, maintenance of appropriate acid balance, and secretion of a variety of hormones. The basic unit of the kidney is the nephron, which consists of a glomerulus and a series of tubules lined by a continuous layer of epithelial cells (Figure 3). The glomerulus consists of mesangial cells and a capillary wall with endothelial cells, glomerular basement membrane, and visceral epithelial cells (podocytes) (Figure 3). Among them, since podocytes play essential role to maintain glomerular filtration barrier, podocyte injury leads to proteinuria and glomerulosclerosis, which are major features of diabetic nephropathy. Podocytes are terminally differentiated cells with a limited proliferative capacity. Therefore, the fate of podocyte depends on its ability to cope with stress. Excess fluid filtered through glomerulus enters urinary space and is reabsorbed by the proximal tubular cells (Figure 3). The proximal tubular cells serve as a system to degrade several molecules reabsorbed from urinary space. Thus, autophagy may be essential to maintain their homeostasis and functions in both podocytes and proximal tubular cells, which might be altered in diabetic condition. If autophagy system is altered in diabetic condition, this alteration of autophagy may be involved in the pathogenesis of diabetic nephropathy.

As expected, autophagy has been identified in both podocytes and proximal tubular cells and is regulated by a variety of stimuli including nutrient stress. Nutrient depletion is the most potent physiological inducer of autophagy, among several that have been reported to regulate autophagy. Here, we show the roles of mTOR, AMPK, and Sirt1, in the regulation of autophagy. The alteration of these pathways is involved in the pathogenesis of several kidney diseases including diabetic nephropathy.

5.1. mTOR. Several studies have shown that hyperactivation of the mTOR pathway in diabetic nephropathy plays a pivotal role in the hypertrophy of existing glomerular and tubular cells [64, 65] and is associated with podocyte injury and the progressive decline of glomerular filtration rates. Other studies have suggested that inhibition of the mTORC1 pathway with rapamycin has renoprotective effects on the progression of diabetic nephropathy in models of type 1 [33] and type 2 diabetes [32, 66–69]. Some reports have shown the additive renoprotective effects of rapamycin treatment including prevention of mesangial expansion and glomerular membrane thickness in type 1 diabetic rats [33] and attenuation of increased glomerular expression of laminin-β1 protein in type 2 diabetic mice [32, 67]. It has been reported that activation of the mTOR pathway is involved in the increased expression of profibrotic cytokines, such as TGF-β1 and connective tissue growth factor, and subsequent interstitial fibrosis in diabetic nephropathy [32–34]. Furthermore, more

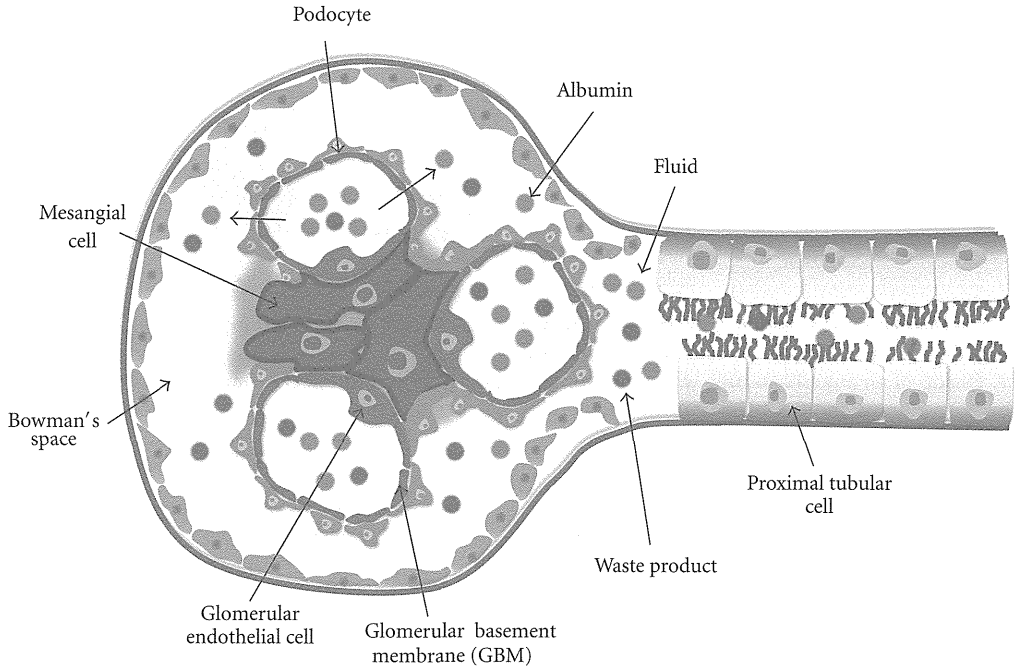

FIGURE 3: *Schematic representation of nephron.* The basic unit of the kidney is the nephron, which consists of a glomerulus and a series of tubules lined by a continuous layer of epithelial cells. The glomerulus consists of mesangial cells and a capillary wall with endothelial cells, glomerular basement membrane, and visceral epithelial cells (podocytes). Fluid containing albumins and waste products is filtered through glomerulus, enters urinary space, and is reabsorbed by the proximal tubular cells.

recent reports have shown that mTORC1 activity is essential to maintain podocyte homeostasis, but its hyperactivation is a causes of glomerular lesion of both type 1 and type 2 diabetic nephropathy [68, 69]. Complete deletion of podocyte mTORC1 activity in podocyte-specific Raptor-deficient mice causes podocyte injury [68]. In contrast, podocyte-specific mTORC1 hyperactivation by podocyte-specific tuberous sclerosis complex (TSC) 1-knockout mice show podocyte injury and glomerular lesion similar to diabetic nephropathy [69]. Finally, podocyte-specific Raptor-heterozygous mice show partial deletion of mTORC1 activity in podocyte and resistance to the development of diabetic nephropathy in both STZ-induced type 1 diabetic mice [68] and type 2 diabetic *db/db* mice [69].

In addition to the above-mentioned function, inhibition of autophagy is a main role of mTOR pathway [44, 58, 70]. Among the several signaling pathways that regulate autophagy in mammalian cells, the classical pathway of serine/threonine kinase, mTOR, plays a major role in the negative regulation of autophagy because it integrates signals that are emitted by growth factors, amino acids, glucose, and energy status [71]. Autophagy is inhibited by the activation of TOR under hypernutrient conditions [44, 58]. The mTOR pathway involves two functional complexes: mTOR complex 1 (mTORC1) and mTORC2. mTORC1, which consists of the mTOR catalytic subunit, regulatory associated protein of mTOR (Raptor), G protein β-subunit-like protein (GβL), proline-rich Akt substrate of 40 kDa (PRAS40), and DEP domain-containing mTOR-interacting protein (Deptor) [72], is sensitive to the immunosuppressant rapamycin [73, 74]. This complex regulates cell growth, metabolism (by integrating amino acid and growth factor signals), energy, and oxygen status [75]. The mTORC1 complex suppresses autophagy via phosphorylation and inactivation of Ulk1, an initiator of autophagosome formation [76]. Although no direct evidences have been provided, hyperactivation of mTOR pathway may suppress autophagy in podocyte and tubular cells in diabetic condition. Furthermore, enhanced activity of autophagy may be involved in the renoprotective effects of rapamycin treatment in diabetic nephropathy. The mTORC2 complex is less sensitive to rapamycin and includes mTOR, rapamycin-insensitive companion of mTOR (Rictor), GβL, stress-activated protein kinase-interacting protein 1 (Sin1), protein observed with Rictor (PROTOR), and Deptor [75, 77]. The mTORC2 complex regulates cytoskeletal organization, metabolism, and cell survival [75, 78, 79]. However, until now, the role of mTORC2 in regulation of autophagy has remained unclear.

5.2. AMPK. AMPK is activated under energy-depleted conditions and is likely to be suppressed in diabetic nephropathy. It has been reported that AMPK is inactivated (decreased phosphorylation of AMPK) in glomeruli and tubules in both type 1 and type 2 diabetic animal models [40, 81–84], which are reversed by agents such as metformin and resveratrol along with attenuation of diabetic glomerular and tubular injury [40, 85, 86]. This introduces the question of how decreases in AMPK activity can be involved in the pathogenesis of diabetic nephropathy. In type 1 and 2 diabetic kidneys, intrarenal lipid metabolism is altered, which is

characterized by enhanced renal lipogenesis and suppressed lipolysis [87–90]. AMPK-mediated phosphorylation inactivates a lipogenic enzyme, acetyl-CoA carboxylase, which results in decreased lipogenesis and enhanced lipolysis [91]. Decreases in renal AMPK activity in these mouse models may be a mechanism of altered renal lipid metabolism and subsequent lipotoxicity-associated renal damage. Since AMPK can affect various cellular metabolism as wells as lipid metabolism [92, 93], the other molecular mechanism should be involved in AMPK-mediated renoprotection.

AMPK plays a central role in the integration of several stress stimuli and is a positive regulator of autophagy in response to nutrient-depleted conditions. AMPK monitors the energy status of the cell by sensing its AMP/ATP ratio [93]. Several upstream kinases, including liver kinase B1 (LKB1), calcium/calmodulin kinase kinase (CaMKII) β, and TGF-β-activated kinase-1 (TAK1), can activate AMPK by phosphorylating a threonine residue on its catalytic α subunit [93]. AMPK can crosstalk with the mTORC1 signal during multiple steps of autophagy regulation. AMPK induces autophagy by inhibiting mTORC1 activity via phosphorylation of its regulatory-associated proteins [44, 58, 94]. Recent studies have shown that AMPK-dependent phosphorylation of Ulk1 induces autophagy [94, 95]. A balance between mTORC1 and AMPK likely directly regulates Ulk1 activity and subsequent autophagy initiation [44]. Thus, in addition to the above-mentioned mechanism, AMPK-mediated induction of autophagy may be involved in its renoprotection. AMPK activation may be linked to autophagy for the maintenance of renal homeostasis in diabetic kidney.

5.3. Sirt1. Sirtuins, the silent information regulator 2 family, were originally identified as NAD^+-dependent deacetylases in experiments in lower species and consist of seven members, Sirt1–Sirt7, in mammals [96, 97]. Sirtuins have been identified as antiaging molecules under calorie-restricted conditions and environmental stress. Some mammalian sirtuins, especially Sirt1, have been shown to play important roles in the regulation of aging, or in the pathogenesis of age-related metabolic diseases such as type 2 diabetes [41, 42, 96]. An increase in the intracellular concentration of NAD^+ by caloric restriction can activate Sirt1. Results that demonstrate the role of Sirt1 in autophagy are still lacking compared with those for mTOR and AMPK, but they have recently been increasing. Sirt1 can deacetylate essential autophagic factors such as Atg5, Atg7, and LC3 [98] and has been shown to induce autophagy. Furthermore, Sirt1 deacetylates the transcription factor Forkhead box O3a (Foxo3a), which leads to enhanced expression of proautophagic BCL2/adenovirus E1V 19-kDa interacting protein 3 (Bnip3) [26].

Renal expression of Sirt1 decreases in type 1 diabetic animal models [99, 100]. Also, reduced forms of nicotinamide adenine dinucleotide (NADH) are metabolites of glucose and fatty acids. Thus, NAD^+/NADH ratios are decreased in cells under conditions where nutrients are in excess, such as diabetes. Sirt1-deacetylase activity should decrease in diabetic nephropathy. Although direct renoprotective effects of Sirt1 in diabetic nephropathy have yet to be elucidated, Sirt1 has shown renoprotective activity in aging kidneys and fibrotic kidney diseases. The previously mentioned findings lead us to speculate that activation of Sirt1 should also have therapeutic efficacy in diabetic nephropathy. Furthermore, Sirt1-induced autophagy activation may contribute to Sirt1-mediated renoprotective effect in diabetic nephropathy.

6. Regulation of Autophagy by Intracellular Stress

Besides nutrient stress, autophagy is upregulated by several intracellular stresses, such as hypoxia, ROS, and ER stress [44]. Based on recent reports, this process is probably a compensatory response to maintain cell integrity. Furthermore, these intracellular stresses have recently been focused on as a pathogenesis of diabetic nephropathy, in addition to the classical pathogenesis of diabetic nephropathy.

6.1. Oxidative Stress. Under conditions where nutrients are in excess, such as diabetes and obesity, the production of ROS in the kidneys is enhanced by high glucose concentrations [101, 102]. Furthermore, high levels of free fatty acids, especially polysaturated fatty acids, also induce ROS production in the kidneys [88, 103]. Oxidative stress is a by-product of mitochondrial respiration and is associated with cell dysfunction. Actually, a recent report has shown abnormal mitochondrial morphology in diabetic kidney [95, 104], suggesting that diabetic kidney fails to remove damaged mitochondria. Thus, restoring the ability to control mitochondria homeostasis should be a therapeutic target of diabetic nephropathy.

Mitochondrial quality control is mediated by mitochondrial autophagy (mitophagy) [105]. Similarly, oxidative stress can induce autophagy to remove damaged mitochondria to protect cells. Thus, autophagy-mediated quality control of mitochondria and subsequent reduction of ROS should be essential to protect kidney in diabetic condition. It has been reported that exogenous hydrogen peroxide activates protein kinase RNA-like ER kinase (PERK), which subsequently phosphorylates eukaryotic initiation factor-2α, activates Atg4, and inhibits mTOR [106]. In response to cellular stress or damage, mitochondrial membranes can be permeabilized. The autophagic recognition of depolarized mitochondria is mediated by a refined voltage sensor, which involves the mitochondrial kinase, phosphatase, and tensin homolog-induced putative kinase 1.

6.2. Hypoxia. In early-stage diabetic nephropathy, hypoxia is aggravated by manifestations of chronic hyperglycemic abnormalities of red blood cells [107, 108], oxidative stress [109], and diabetes mellitus-induced tubular apoptosis; as such, tubulointerstitial hypoxia in diabetes mellitus might be an important early event.

Hypoxia is also a stimulatory factor of autophagy. Hypoxia-induced autophagy largely depends on hypoxia-inducible factor-1α (HIF-1α), which is a transcription factor that is activated and stabilized under hypoxic conditions

Table 1: Autophagy-related kidney diseases.

Species and methods to monitor autophagy	Disease model	Effects of autophagy	Reference
Sprague-Dawley rats, immunohistochemistry of LC3 and Western blotting of LC3-II	Cyclosporine A-induced nephrotoxicity	Protection against tubular cell death	[80]
C57BL/6 mice, EM, and Western blotting of LC3-II	Cisplatin injury	Protection against tubular cell death	[30]
C57BL/6 mice, EM, immunofluorescence of LC3, and Western blotting of LC3-II	Aging	Protection against aging and hypoxia-related tubular damage	[26]
GFP-LC3 mice	Cisplatin injury	Protection against tubular cell death	[31]
C57BL/6 mice, EM, and Western blotting of LC3-II with 3-MA and chloroquine	Ischemia reperfusion	Protection against tubular cell death	[28]
Proximal tubular epithelial cell-specific Atg5-deficient mice	Ischemia reperfusion	Protection against tubular cell death	[29]
Podocyte-specific Atg5-deficient mice	Aging, protein overload-, LPS-, PAN-, and adriamycin-induced glomerular injury	Protection against podocyte injury	[27]

EM: electron microscopy; GFP: green fluorescent protein; 3-MA: 3-methladenine; Atg: autophagy-related genes; LPS: lipopolysaccharide; PAN: puromycin aminonucleoside.

[110, 111]. HIF-1α activates transcription of Bnip3 and Bnip3L and subsequently induces autophagy. Normally, Beclin 1 interacts with Bcl-2 proteins. Bnip3 can disrupt this interaction, liberating Beclin 1 from Bcl-2 in cells and leading to autophagy. Thus, HIF1α-induced Bnip3 overexpression promotes autophagy [112]. The transcription of Bnip3 is also upregulated by the transcription factor FOXO3, which is deacetylated and positively regulated by Sirt1 [26]. Hypoxia causes damage to the mitochondria and intracellular accumulation of ROS [113]. Removing the damaged mitochondria under hypoxic conditions is also an important role of Bnip3-mediated autophagy. Thus, to investigate whether hypoxia-induced and Sirt1-mediated autophagy is altered in diabetic kidney is interesting. If it is altered, to restore autophagy activity even under diabetic condition should be important to protect kidney form hypoxia.

6.3. ER Stress. ER stress has recently been focused as a pathogenesis of diabetic nephropathy. The induction of ER stress and subsequent apoptosis by hyperglycemia and high levels of free fatty acids (polysaturated fatty acids) are observed in podocytes [114]. Additionally, in proteinuric kidney diseases, including diabetic nephropathy, massive proteinuria filtered from glomeruli causes ER stress responses and subsequent apoptosis in renal tubular cells [14, 115]. Thus, to suppress inadequate ER stress is thought as a therapeutic strategy of diabetic nephropathy.

It is known that ER stress as well as hypoxia and ROS also cause autophagy. The ER is not only involved in protein synthesis and maturation but may also constitute a major source/scaffold for the autophagic isolation membrane [47]. When misfolded proteins are not exported efficiently to the cytoplasm and accumulate in the ER, the unfolded protein response (UPR) is often induced [116–118]. The UPR consists of three main branches that are controlled by the ER membrane proteins: PERK; activating transcription factor-6 (ATF6); inositol requiring enzyme 1 (IRE1) [116–118]. Among these UPR-related proteins, PERK and ATF6 have been reported to induce autophagy [44]. PERK induces the transcriptional activation of LC3 and Atg5 through the action of the transcription factors ATF4 and CCAAT-enhancer-binding protein homologous protein, respectively [119]. It has been suggested that IRE1 is also involved in the induction of autophagy by phosphorylation of Beclin 1 via c-Jun NH2-terminal kinase-1 [44]. Enhanced and prolonged ER stress causes several pathogenic features such as apoptosis and inflammation [117, 118]. Thus, autophagy-mediated ER degradation (ERphagy) may be required for cell protection from prolonged cytotoxic ER stress shown in diabetic kidney.

7. Autophagy in the Kidneys

The study of autophagy has previously been undertaken in lower species. The study of autophagy in mammalian systems is advancing rapidly and has revealed that mammalian autophagy is involved in the pathogenesis of various metabolic or age-related diseases [18–27].

Recently, nephrologists have also entered this exciting field of study. In this section, we review recent studies on the pathophysiology of autophagy in the kidneys. Autophagy has been observed in various parts of the kidneys, including proximal tubules, and thick ascending limbs. In particular, in podocytes, higher levels of constitutive autophagy have been

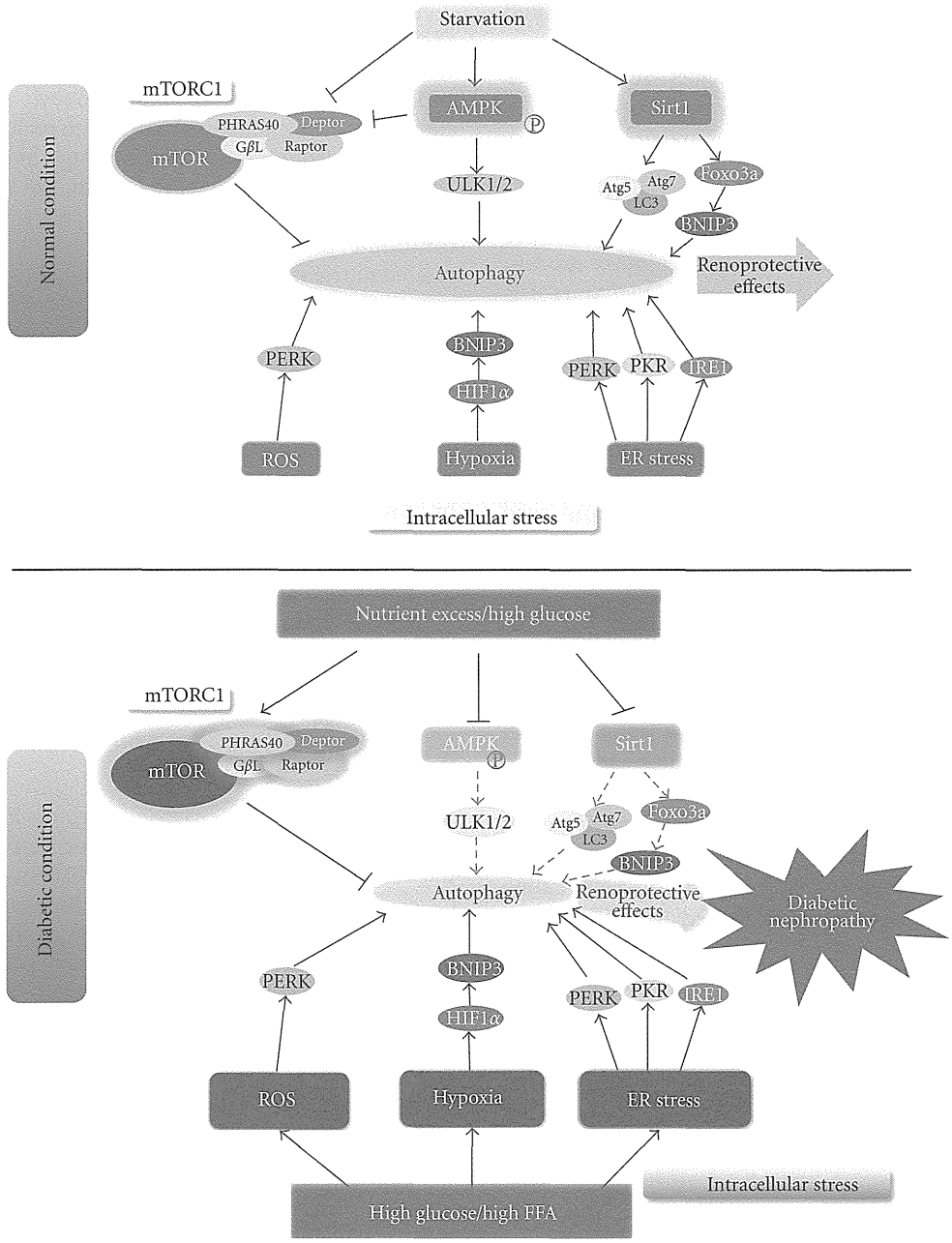

FIGURE 4: *Regulation of autophagy by nutrient and intracellular stresses and the relationship between autophagy and the progression of diabetic nephropathy.* Under normal conditions, intracellular stresses such as hypoxia, mitochondrial ROS, and ER stress induce autophagy. Nutrient depletion enhances autophagy by inhibiting mTORC1 and by activating AMPK and Sirt1. This activation of autophagy helps to maintain intracellular homeostasis and may have renoprotective effects. In contrast, under diabetic conditions, high glucose or FFA levels increase intracellular stresses, leading to the progression of diabetic nephropathy. Furthermore, nutrient excess and high glucose levels under diabetic conditions inhibit autophagy by inhibiting AMPK and Sirt1, and by activating mTORC1. This inactivation of autophagy may reinforce the progression of diabetic nephropathy. ROS: reactive oxygen species; ER: endoplasmic reticulum; mTORC1: mammalian target of rapamycin (mTOR) complex 1 (mTORC1); AMPK: AMP-activated protein kinase; FFA: free fatty acid.

observed using GFP-LC3 transgenic mice even under normal conditions [27]. As for the role of autophagy in renal pathophysiology, several researchers have reported the significance of autophagy in experimental renal injury models. In several experimental animal models of glomerulonephritis, including puromycin aminonucleoside and adriamycin-induced proteinuria, autophagy has been identified and shown to play renoprotective and antiproteinuric roles in podocytes through the use of podocyte-specific Atg5 knockout mice [27]. It has been recently reported that the normal aging process suppresses autophagy in podocytes, and that podocyte-specific deletion of *Atg5* leads to glomerulopathy in aging

mice that is accompanied by accumulation of oxidized and ubiquitinated proteins, ER stress, and proteinuria [27].

In renal tubules as well as in podocytes, autophagy has been reported to play a renoprotective role under several pathological conditions. In renal ischemia-reperfusion injury models, the upregulation of autophagy to protect the kidneys was observed using 3-MA, chloroquine [28], and proximal tubular epithelial cell-specific Atg5 knockout mice [29]. Additionally, in cisplatin-induced acute kidney injury models, the increase of autophagosomes was observed by EM, LC3-II Western blotting [30], and GFP-LC3 transgenic mice [31]. Hypoxia is one of the causes of renal tubular damage in aged kidney [120]. We have previously shown that hypoxia-induced autophagy activity declined with age, which led to accumulations of damaged mitochondria and mitochondrial ROS in the kidney [26]. Interestingly, long-term calorie restriction (CR) restored autophagy activity even in aged kidney [26]. As a mechanism, Sirt1-mediated autophagy was essential in CR-mediated renoprotection in aged kidney [26]. Bnip3 expression is essential to induce autophagy under hypoxic condition [121] and is positively regulated by a transcriptional factor Foxo3a [122]. This regulation was altered in aged kidney. On the other hand, CR-mediated Sirt1 activation deacetylated and activated Foxo3a transcriptional activity and subsequent Bnip3-mediated autophagy even in aged kidney [26]. Furthermore, the kidney of heterozygous Sirt1-knockout mice showed lower autophagy activity along with the decrease in Bnip3 expression, and thus they were resistant to CR-mediated antiaging effects [26]. This finding suggests that Sirt1 is essential for CR-mediated renoprotection.

Thus, accumulative evidence has demonstrated the pathophysiological importance of autophagy in the kidneys (Table 1). However, the role and existence of autophagy in other types of renal cells besides podocytes and proximal tubular cells is not known.

8. Perspective

It is evident that the above-mentioned nutrient-sensing signals exist in the kidneys. However, what are their physiological roles in this organ? The kidneys require sufficient amounts of ATP for maintenance of their functions and avidly consume oxygen to drive mitochondrial oxidative phosphorylation among major organs. A small percentage of oxygen consumed by mitochondria is incompletely reduced to ROS, and this unremitting generation of oxidants during mitochondrial respiration, albeit in small amounts, may cumulatively damage the kidneys, which are heavily dependent on mitochondrial metabolism. Regulating mitochondrial metabolism in response to nutrient conditions via regulation of autophagy that can remove damaged mitochondria and subsequent ROS may be a physiological role of renal nutrient-sensing signals.

Autophagy is regulated by nutrient conditions, and its alteration associates with various metabolic and age-associated diseases. Although studies on autophagy have methodological limitations, as outlined above, it is evident that autophagy deficiency is associated with podocyte and tubular cell injuries from the studies using Atg5-knockout mice [27, 29]. These findings lead us to hypothesize that autophagy is altered in diabetic kidneys, and autophagy deficiency should contribute to the pathogenesis of diabetic nephropathy. Altered nutrient-sensing signals in diabetic kidneys may contribute to accumulation of mitochondrial ROS via suppression of autophagy, which may be associated with initiation of the early stages of diabetic nephropathy. Both hypoxia and proteinuria-induced ER stress contribute to proximal tubular cell damage in the progressive and overt stages of diabetic nephropathy. Why do diabetic kidneys show a weakness against these stresses? How can we protect the kidneys from these stresses even under diabetic conditions? One answer may be derived from autophagy studies. Autophagy deficiency in diabetic kidneys may make tubular cells fragile under hypoxic and ER stress and possibly lead to progression of diabetic nephropathy (Figure 4). Activation of autophagy may be a therapeutic option for the advanced stages of diabetic nephropathy.

9. Concluding Comments

In recent decades, numerous investigators have been making efforts to identify the molecular mechanisms involved in the initiation and progression of diabetic nephropathy to develop new therapeutic strategies. However, end-stage renal disease due to diabetic nephropathy continues to increase worldwide. There is an urgent need to identify additional new therapeutic targets for prevention of diabetic nephropathy. We have provided a perspective on whether autophagy is involved in the pathogenesis of diabetic nephropathy and whether it is an acceptable new therapeutic target. Unfortunately, there have still not been many studies that have focused on autophagy in diabetic nephropathy. In the next few years, studies using Atg-gene knockout/knockdown mice combined with different methodologies will elucidate this possibility. Finally, these studies will ultimately give us a clearer perspective as to whether autophagy should be considered as a novel therapeutic target to halt the progression of diabetic nephropathy.

Acknowledgment

This study was supported in part by a Grant-in-Aid For Diabetic Nephropathy Research, from the Ministry of Health, Labour and Welfare of Japan.

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Environ Health Prev Med (2013) 18:16–23
DOI 10.1007/s12199-012-0283-9

REGULAR ARTICLE

Decreased activity of daily living produced by the combination of Alzheimer's disease and lower limb fracture in elderly requiring nursing care

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Received: 11 March 2012 / Accepted: 18 April 2012 / Published online: 11 May 2012

Abstract
Objectives Alzheimer's disease (AD) impairs cognitive functions, subsequently decreasing activity of daily living (ADL), and is frequently accompanied by lower limb fracture including hip fracture in the elderly. However, there have been few studies on what kinds of physical functions are affected or what degrees of dysfunction are produced by this combination. This study aims to clarify the relationship between decreased ADL and the combination of AD and lower limb fracture.
Methods We examined present illness and ADL in 4340 elderly aged 82.8 ± 9.36 years [average ± standard deviation (SD)] requiring nursing care and compared ADL between elderly with and without AD or lower limb fracture treated with surgery or conservatively using analysis of covariance (ANCOVA), with age and sex as covariants.
Results We recognized that activities of cognitive function ($p < 0.001$), eating (dysphagia) ($p < 0.001$), eating (feeding) ($p < 0.001$), and toilet use ($p < 0.001$) in the elderly with AD were significantly lower than in those without the disease, even after adjusting for sex and age. Activities of bed mobility ($p < 0.05$), transfer and locomotion ($p < 0.001$), and bathing ($p < 0.05$) in the elderly with a fracture treated with surgery were significantly lower, which differed from the results of AD. Significant interactions of AD and fracture treated with surgery on the ADL scores for bed mobility ($p < 0.001$), dysphagia ($p < 0.01$), feeding ($p < 0.001$), and toilet use ($p < 0.05$) show that the combination had a much more profound influence on the ADL scores than AD or fracture alone. We obtained almost the same results for fractures treated conservatively as for fractures treated with surgery.
Conclusions These results demonstrated that the combined effects of AD and lower limb fracture were significantly greater than expected additive effects of AD and fracture, suggesting that the combination of AD and lower limb fracture has synergistic effects on almost all types of ADL except cognitive functions.

Keywords Alzheimer's disease · Hip fracture · Elderly · Activity of daily living · Physical function

Introduction

Alzheimer's disease (AD) is the most common form of dementia. The number of individuals with AD has been increasing considerably in recent years, accounting for more than 50 % of dementia cases [1]. AD causes cognitive impairment of elderly in need of care, consequently interfering with daily tasks and decreasing activities of daily living (ADL) in many ways [2]. It is known that patients with AD suffer from various kinds of complications [3]. The increased risk of falling associated with AD leads to lower limb fracture including hip fracture [4]. In addition, taken together with the fact that AD and osteoporosis have common risk factors [5, 6], the incidence of fracture is higher in elderly with AD. Thus, lower limb fractures could accompany AD as one of its complications.

To make matters worse, elderly made bedridden by the aftereffects of lower limb fracture could develop cognitive impairment as its complication [7]. Even if an elderly person

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is not made bedridden, the aftereffects of fracture sometimes impair cognitive functions, subsequently decreasing basic ADL [8–10]. AD and fracture, which are commonly encountered in the elderly, aggravate each other, being the two major factors subsequently deteriorating ADL in bedridden elderly. Therefore, AD and fracture produce a vicious spiral, resulting in not only cognitive impairments but also deterioration in ADL [3, 5]. However, it remains to be elucidated exactly what kinds of physical functions are affected and to what degree ADL is changed by the combination of AD and lower limb fracture. To clarify this, we performed a national survey in nursing care institutions in Japan, examining the decreased ADL in elderly with and without AD and lower limb fracture.

Subjects and methods

Subjects

Three hundred and ninety facilities were randomly selected out of 3410 nursing care institutions in Japan. Ten resident patients and 10 daycare patients were sampled at random from each facility. A total of 7800 patients were prospective subjects in this study. We mailed a questionnaire to caregivers in each facility inquiring about ADL and medical conditions, including AD and fracture, as described below and obtained 4340 responses (55.6 %, 2132 resident patients and 2208 daycare patients). This survey was conducted from December 2009 to February 2010. The average age and SD of the 4340 subjects were 82.8 and 9.36 years. This research was conducted after obtaining approval from the ethical committee of the Japan National Conference of Geriatric Health Care Facility.

Questionnaire

The questionnaires regarding medical conditions in subjects were related to AD and lower limb fracture (hip fracture and lower leg fracture) requiring surgery, and fractures treated without surgery. To examine AD, the questions concerned neuropsychological examinations including the patients' medical history, neurological testing, Mini-Mental State Examination [11], and standard clinical evaluation including brain scanning. In the diagnostic process, DSM-III [12] was used for diagnosis and to rule out other factors. The National Institute of Neurological and Communicative Disorders and Stroke–Alzheimer's Disease and Related Disorders Association guidelines [13] were used for detection and diagnosis of either possible or probable AD. Lower limb fracture was diagnosed after an appropriate procedure including X-ray roentgenogram. The diagnoses of AD and fracture were performed by a physician of the facility within 1 year prior to the contact with nursing care institutions for elderly in this study. Our subjects included 491 patients with possible or probable AD, 405 and 196 patients with fracture treated with surgery and conservatively, respectively, and 3248 elderly without AD or fracture.

Regarding ADL, the questionnaire was developed on the basis of a standard described previously [14, 15]. They dealt with bed mobility, transfer and locomotion, cognitive function (orientation), cognitive function (communication), cognitive function (mental activity), eating (dysphagia), eating (feeding), toilet use, and bathing for elderly requiring both residential nursing care and daycare. Each ADL was categorized into a five-point scale. In the case of bed mobility, for example, a score of 5 represents being able to stand on one's feet and maintain this posture; score 4: having difficulty maintaining a standing posture, but being able to transfer from one place to another in a sitting position; score 3: being unable to move in a sitting position, but being able to sit in a proper posture without support; score 2: having difficulty sitting in an upright posture, but being able to turn over on a bed; and score 1: not being able to turn over on a bed. In the case of eating (feeding), a score of 5 represents being able to eat well without any support of others; score 4: spilling food during eating; score 3: having difficulty eating by themselves, but being able to eat with support for their posture and position of the dish; score 2: being unable to eat without complete support; and score 1: being unable to eat with any support (receiving tube feeding). Thus, the requirement for more concentrated nursing care during eating for the elderly decreased the ADL score. A lower score for each ADL implies worse ADL. In Table 1, we show ADL and prevalence of AD, fracture treated with surgery, and fracture treated conservatively according to the status of nursing care (residential and daycare).

Statistics

Pearson's correlation coefficients of age showed weak but significant correlations with bed mobility (−0.118), transfer and locomotion (−0.145), cognitive function (orientation) (−0.262), cognitive function (communication) (−0.199), cognitive function (mental activity) (−0.232), eating (dysphagia) (−0.142), eating (feeding) (−0.088), toilet use (−0.126), and bathing (−0.077) (all $p < 0.001$). Because of significant differences in proportions in terms of sex and age between elderly with and without diseases in addition to significant correlations between age and ADL scores, we compared each ADL score by analysis of covariance (ANCOVA) with age and sex as covariants, in which age was assigned as a continuous variable and sex was dummy-coded as follows: male 0, female 1. Mean

Table 1 Activities of daily living and prevalence of AD, fracture treated with surgery, and fracture treated conservatively according to status of nursing care (residential and daycare)

Status	Number	Proportion of women (%)	Age (years; mean ± SD)	Activities of daily living (age-adjusted score; mean ± SD)							
				Bed mobility	Transfer and locomotion	Cognitive function (orientation)	Cognitive function (communication)	Cognitive function (mental activity)	Eating (dysphagia)	Eating (feeding)	Toilet use	Bathing
Residential	2132	79.7	85.2 ± 8.34	3.27 ± 1.45	2.07 ± 0.72	3.31 ± 1.33	3.24 ± 1.36	3.25 ± 1.34	3.84 ± 1.28	4.01 ± 1.25	3.22 ± 1.26	2.83 ± 0.69
Daycare	2208	57.3	80.5 ± 9.20	4.24 ± 1.05	2.74 ± 0.99	4.25 ± 1.03	4.19 ± 1.12	4.22 ± 1.09	4.66 ± 0.80	4.64 ± 0.74	4.23 ± 1.06	3.35 ± 0.91

Status	Number	Proportion of women (%)	Age (years; mean ± SD)	Prevalence (age-adjusted score)		
				AD	Fracture (surgery)	Fracture (conservatively)
Residential	2132	79.7	85.2 ± 8.34	17.0	12.2	5.53
Daycare	2208	57.3	80.5 ± 9.20	5.80	6.57	3.53

ADL score and standard deviation adjusted by age and sex were calculated using regression coefficients corresponding to age, sex, and disease obtained by ANCOVA and raw mean values of age and sex in total subjects. To assess the interaction for ADL score between the combination of AD and fracture treated with or without surgery, we used two-way ANCOVA with age and sex as covariants. The statistical software SPSS version 17 was used. Two-tailed p-values less than 5 % were considered statistically significant.

Results

Changes in ADL with AD and fracture

The proportion of women and age of subjects with AD were significantly higher than those without AD. The activities of cognitive function (orientation), cognitive function (communication), cognitive function (mental activity), eating (dysphagia), eating (feeding), and toilet use in the elderly with AD were significantly lower than those without the disease, even after controlling for sex and age (Table 2). Activities of bed mobility, transfer and locomotion, and bathing in elderly with a fracture requiring surgical operation were significantly lower, which were quite different results from AD (Table 3). Table 4 also shows lower scores on bed mobility, transfer and locomotion, and bathing of the patients with a fracture treated without surgery.

The interaction between AD and fracture

The two-way ANCOVA demonstrated a significant main effect of AD on ADL scores of all cognitive functions and eating functions, as well as toilet use, and a significant main effect of fracture on all scores of ADL except cognitive functions. Furthermore, we recognized significant interactions between AD and fracture on the activities of bed mobility, eating (dysphagia), eating (feeding), and toilet use after adjustment for age and sex (Table 5). Table 6 shows ADL scores in the cases of patients with complications of AD and fracture treated conservatively. ADL scores of all cognitive functions and eating functions, as well as toilet use, in the elderly with AD were significantly decreased compared with those without AD. The scores of all ADL except cognitive functions and eating functions were significantly decreased compared with those without fracture treated conservatively. Interactions between ADL and fracture treated conservatively were recognized for the scores of bed mobility, transfer and locomotion, and toilet use even after adjusting for sex and age. There were decreases in the scores of eating (dysphagia), eating

Table 2 Comparisons of activities of daily living in patients with and without AD

AD	Number (proportion of women, %)	Age (years; mean ± SD)	Activities of daily living (age- and sex-adjusted score; mean ± SD)								
			Bed mobility	Transfer and locomotion	Cognitive function (orientation)	Cognitive function (communication)	Cognitive function (mental activity)	Eating (dysphagia)	Eating (feeding)	Toilet use	Bathing
(−)	3849 (67)	82.5 ± 9.56	3.76 ± 1.30	2.41 ± 0.87	3.92 ± 1.18	3.83 ± 1.24	3.86 ± 1.18	4.28 ± 1.12	4.36 ± 1.05	3.77 ± 1.24	3.10 ± 0.81
(+)	491 (82)	85.3 ± 7.07	3.78 ± 1.35	2.44 ± 0.91	2.77 ± 1.17	2.85 ± 1.26	2.84 ± 1.24	4.07 ± 1.13	4.09 ± 1.06	3.46 ± 1.26	3.03 ± 0.84
Statistics	***	***			***	***	***	***	***	***	

ANCOVA was used to detect significant difference in the score in elderly with AD compared with the corresponding one in elderly without AD after adjustment for age and sex, *** $p < 0.05$. In the analysis of the difference in age and proportion between sexes, Student's t test and χ^2 test were used, respectively; *** $p < 0.001$

Table 3 Comparisons of activities of daily living in patients with and without fracture treated with surgery

Fracture	Number (proportion of women, %)	Age (years; mean ± SD)	Activities of daily living (age- and sex-adjusted score; mean ± SD)								
			Bed mobility	Transfer and locomotion	Cognitive function (orientation)	Cognitive function (communication)	Cognitive function (mental activity)	Eating (dysphagia)	Eating (feeding)	Toilet use	Bathing
(−)	3935 (66)	82.3 ± 9.40	3.78 ± 1.32	2.43 ± 0.88	3.79 ± 1.19	3.73 ± 1.25	3.75 ± 1.25	4.26 ± 1.13	4.33 ± 1.07	3.74 ± 1.25	3.10 ± 0.82
(+)	405 (89)	87.4 ± 7.45	3.60 ± 1.35	2.24 ± 0.93	3.77 ± 1.25	3.66 ± 1.33	3.74 ± 1.29	4.26 ± 1.15	4.32 ± 1.09	3.67 ± 1.27	3.00 ± 0.85
Statistics	***	***	*	***							*

ANCOVA was used to detect significant difference in the score in elderly with fracture treated with surgery compared with the corresponding one in elderly without fracture treated with surgery after adjustment for age and sex, * $p < 0.05$, *** $p < 0.001$. In the analysis of the difference in age and proportion between sexes, Student's t test and χ^2 test were used, respectively; *** $p < 0.001$

Table 4 Comparisons of activities of daily living in patients with and without fracture treated conservatively

Fracture	Number (proportion of women, %)	Age (years; mean ± SD)	Activities of daily living (age- and sex-adjusted score; mean ± SD)								
			Bed mobility	Transfer and locomotion	Cognitive function (orientation)	Cognitive function (communication)	Cognitive function (mental activity)	Eating (dysphagia)	Eating (feeding)	Toilet use	Bathing
(−)	4144 (68)	82.7 ± 9.41	3.77 ± 1.29	2.42 ± 0.90	3.79 ± 1.22	3.72 ± 1.29	3.74 ± 1.22	4.26 ± 1.09	4.33 ± 1.03	3.74 ± 1.22	3.10 ± 0.84
(+)	196 (83)	85.3 ± 7.79	3.56 ± 1.33	2.25 ± 0.91	3.87 ± 1.23	3.70 ± 1.30	3.80 ± 1.27	4.18 ± 1.12	4.35 ± 1.06	3.63 ± 1.25	2.96 ± 0.84
	***	***	*	*							*

ANCOVA was used to detect significant difference in the score in elderly with fracture treated conservatively compared with the corresponding one in elderly without fracture treated conservatively after adjustment for age and sex, * $p < 0.05$. In the analysis of the difference in age and proportion between sexes, Student's t test and χ^2 test were used, respectively; *** $p < 0.001$

Table 5 Comparisons of activities of daily living in patients with and without AD and fracture treated with surgery

Disease	Number (proportion of women, %)	Age (years; mean ± SD)	Activities of daily living (age- and sex-adjusted score; mean ± SD)								
			Bed mobility	Transfer and locomotion	Cognitive function (orientation)	Cognitive function (communication)	Cognitive function (mental activity)	Eating (dysphagia)	Eating (feeding)	Toilet use	Bathing
(−)	3495 (64)	82.0 ± 9.61	3.77 ± 1.30	2.43 ± 0.89	3.93 ± 1.18	3.84 ± 1.24	3.86 ± 1.24	4.28 ± 1.12	4.35 ± 1.06	3.77 ± 1.24	3.11 ± 0.83
AD	440 (81)	84.9 ± 7.09	3.86 ± 1.34	2.48 ± 0.90	2.77 ± 1.17	2.88 ± 1.26	2.85 ± 1.24	4.12 ± 1.11	4.15 ± 1.07	3.51 ± 1.26	3.06 ± 0.84
Fracture	354 (88)	87.2 ± 7.63	3.69 ± 1.35	2.27 ± 0.92	3.89 ± 1.19	3.79 ± 1.28	3.85 ± 1.24	4.34 ± 1.13	4.42 ± 1.07	3.75 ± 1.26	3.02 ± 0.85
Combination	51 (94)	88.5 ± 5.93	3.01 ± 1.34	2.05 ± 0.91	2.74 ± 1.18	2.65 ± 1.26	2.78 ± 1.24	3.63 ± 1.12	3.60 ± 1.06	3.05 ± 1.25	2.81 ± 0.84
Main effect of AD					***	***	***	***	***		
Main effect of fracture			***	***				*	**	*	*
Interaction			***					**	***	*	

Two-way ANCOVA with age and sex as covariants was used in the analysis of the difference in ADL score, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. The sex and age distributions among the four groups were recognized to be significantly different (both $p < 0.001$) by χ^2 test and one-way analysis of variance (ANOVA), respectively

Table 6 Comparisons of activities of daily living in patients with and without AD and fracture treated conservatively

Disease	Number (proportion of women, %)	Age (years; mean ± SD)	Activities of daily living (age- and sex-adjusted score; mean ± SD)								
			Bed mobility	Transfer and locomotion	Cognitive function (orientation)	Cognitive function (communication)	Cognitive function (mental activity)	Eating (dysphagia)	Eating (feeding)	Toilet use	Bathing
(-)	3676 (68)	82.34 ± 9.61	3.77 ± 1.33	2.42 ± 0.91	3.92 ± 1.15	3.83 ± 1.21	3.86 ± 1.21	4.29 ± 1.09	4.36 ± 1.03	3.77 ± 1.21	3.11 ± 0.85
AD	468 (82)	85.15 ± 7.10	3.82 ± 1.34	2.46 ± 0.91	2.77 ± 1.17	2.86 ± 1.28	2.84 ± 1.23	4.09 ± 1.12	4.11 ± 1.06	3.50 ± 1.25	3.05 ± 0.84
Fracture	173 (83)	85.05 ± 7.96	3.66 ± 1.33	2.30 ± 0.91	3.99 ± 1.17	3.83 ± 1.26	3.90 ± 1.24	4.25 ± 1.12	4.41 ± 1.07	3.74 ± 1.25	2.99 ± 0.84
Combination	23 (78)	87.22 ± 6.16	2.86 ± 1.34	1.89 ± 0.91	2.80 ± 1.17	2.64 ± 1.27	2.87 ± 1.23	3.64 ± 1.12	3.82 ± 1.06	2.73 ± 1.25	2.68 ± 0.84
Main effect of AD					***	***	***	***	***	***	
Main effect of fracture			**	***						**	*
Interaction			*	*						**	

Two-way ANCOVA with age and sex as covariants was used in the analysis of the difference in ADL score, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. The sex and age distributions among the four groups were recognized to be significantly different (both $p < 0.001$) by χ^2 test and one-way ANOVA, respectively

(feeding), and bathing in the patients with the combination, but these were not statistically significant (Table 6).

Discussion

Decreased ADL in eating (dysphagia), eating (feeding), and toilet use as well as cognitive functions, but not in bed mobility, or transfer and locomotion, were recognized in AD patients in this study. Although AD in our subjects was not considered to produce symptoms severe enough to affect motor functions, the influences of AD on eating (dysphagia), eating (feeding) [16, 17], and toilet use [18] seemed to be associated with cognitive impairments due to AD. These findings are supported by epidemiological studies on the natural course of the progress of AD [19]. In contrast to the results for AD, the patients with fracture treated either with or without surgery showed decreased ADL scores in bed mobility, transfer and locomotion, and bathing. Motor dysfunctions including difficulties in bathing are common following a hip fracture or a lower limb fracture [20, 21]. Thus, many epidemiological studies have evaluated effects of AD or lower limb fracture on individual ADL in the elderly, albeit separately.

The kinds of function affected and the degrees of change in ADL in patients suffering from the combination of AD and lower limb fracture are clarified by the observation that the kinds of dysfunction for AD and fracture were quite different. Our results showed significant interactions on the ADL scores of bed mobility, eating (dysphagia), eating (feeding), and toilet use, demonstrating that the combination of AD and fracture treated with surgery has more profound influences on the ADL scores than AD or fracture alone. This implies that the combined effect was significantly greater than the expected additive effect of AD and fracture. The decreased ADL score of transfer and locomotion with this combination was not recognized as involving a significant interaction between AD and fracture with surgery, but it was in cases without surgery. Thus, the combined effects of AD and lower limb fracture on ADL excluding cognitive functions and bathing were much larger than the sum of the sole effects of AD and fracture. The lack of an interaction for cognitive functions seems to be due to severe influences of AD on cognitive functions, for which complication with fracture did not bring any further deterioration [2, 3].

A fracture in a lower limb has a much greater influence on toilet use because this ability largely depends upon the functions of the lower limbs [18]. However, there are few studies demonstrating how the complication of lower limb fracture with AD affects dysphagia or eating activity, which is unaffected by the fracture alone [22]. Our findings

showing that fracture in a lower limb aggravates dysfunctions in eating (dysphagia) and eating (feeding) produced by AD suggest that the fracture makes AD worse and more progressive. This finding is supported by many studies [23–25], which have shown that a lower limb fracture affects the central nervous system through motor dysfunction, resulting in cognitive impairment.

On the other hand, our finding showing that the activities of bed mobility, and transfer and locomotion that were decreased by the fracture were further deteriorated by its complication with AD supports the idea that AD has greater influence on motor functions than expected. It is well known that osteoporosis, which often accompanies AD, leads to motor dysfunctions [5]. Some studies have demonstrated that problems in abnormal behaviors in AD patients may produce muscular dystrophy, which further damages physical functions [18, 25]. Taking these findings together, motor dysfunctions such as in bed mobility, and transfer and locomotion are considered to be accelerated by the complication of fracture with AD.

Women are at higher risk of developing AD and fracture [26, 27]. Our results showed that women account for 94 % of patients with the complications of AD and fracture. Therefore, the synergistic effects of AD and lower limb fracture on ADL seen in this study explain well that ADL in all fields in elderly women are lower than those in elderly men of the same age.

There were few differences in decreased ADL between cases of fracture treated with and without surgery. Our data regarding fracture treated with and without surgery do not coincide well with the findings that patients with fracture treated with surgery show better prognosis in terms of ADL than those treated without surgery [7, 10]. We did not obtain any data regarding social circumstances and physical background under which the patients with fracture did not receive surgery. Analysis of the data might confirm effects of surgery on the recovery from fracture, which was not seen in this study. Since this study was performed with a cross-sectional design, the causal effects of AD and fracture on ADL must be demonstrated by further follow-up study.

In conclusion, our results showed that the combination of AD and lower limb fracture has more profound influence on ADL scores in terms of bed mobility, transfer and locomotion, eating (dysphagia), eating (feeding), and toilet use, demonstrating that the combined effects were significantly greater than expected additive effects of AD and fracture, suggesting that the complication of AD with lower limb fracture has synergistic effects on almost all types of ADL besides cognitive functions. The interaction between AD and fracture on ADL seemed to be due to effects of both AD on motor function and fracture on central nervous functions.

Acknowledgments The work was funded by a Grant-in-Aid for Scientific Research (B) from the Ministry of Health, Labor, and Welfare (2009–2010), Japan.

Conflict of interest None.

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