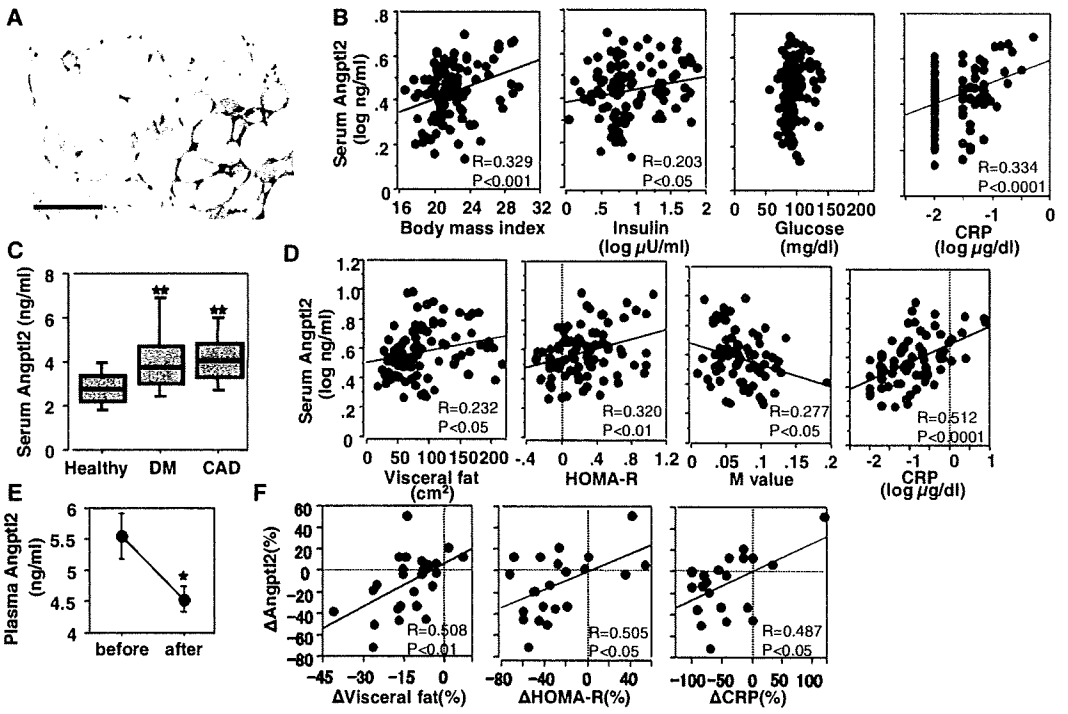

Figure 2. Circulating Angptl2 Is Correlated with Adiposity, Insulin Resistance, and Inflammation in Humans

(A) Immunohistochemical staining for Angptl2 in human adipose tissue. Scale bar, 100 μm.

(B) Correlation of the serum Angptl2 level with the body mass index or serum insulin, glucose, and CRP levels in healthy volunteers (n = 98).

(C) Serum Angptl2 levels in healthy volunteers (Healthy, n = 98) and in patients with type 2 diabetes (DM, n = 89) or coronary artery disease (CAD, n = 109). Horizontal bars represent the 10%–90% percentile range, and boxes indicate the 25%–75% percentile range. The horizontal line in each box corresponds to the median.

(D) Correlation of the serum Angptl2 level with the visceral fat area, HOMA-R index, M value, and CRP level in diabetic patients.

(E and F) Changes of the plasma Angptl2 level in obese diabetic male patients after pioglitazone treatment (n = 27). Plasma Angptl2 levels (mean ± SEM) before and after treatment (E). Correlation of the change (%) of the plasma Angptl2 level with the change (%) of the visceral fat area, subcutaneous fat area, HOMA-R index, and CRP level. Correlation coefficient (R) and probability (P) values are shown (F). *p < 0.05 and **p < 0.01 compared with controls.

a result, both the cellular Angptl2 mRNA level and its protein concentration in the culture medium were significantly increased (Figures 1D and 1E, Figure S2, and data not shown). Angptl2 mRNA in mesenteric white adipose tissue and serum Angptl2 protein levels were increased in obese mice fed a high-fat diet (Figures 1F and 1G), suggesting that Angptl2 is a bioactive adipocyte-derived factor that has a role in obesity and related metabolic diseases.

Circulating Angptl2 Level Is Correlated with Adiposity, Systemic Insulin Resistance, and Inflammation in Humans

Immunohistochemical analysis revealed that Angptl2 was expressed by the adipocytes of human adipose tissue (Figure 2A). We analyzed the circulating levels of Angptl2 in various human subjects by using an enzyme-linked immunosorbent assay (ELISA). In healthy normal-weight volunteers aged from 20 to 59 years, the serum Angptl2 concentration ranged from 1.36 to 4.98 ng/ml, and the distribution was normal after log transformation (Figure S3A). Plasma levels were comparable and strongly correlated with the corresponding serum levels (Figure S3B). There was no significant difference of serum Angptl2 concentration between genders (data not shown). Angptl2 level showed a positive correlation with body mass index, serum insulin level, and serum C-reactive protein (CRP) level. In contrast, the level of Angptl4, which has already been identified as an adipocyte-derived Angptl, showed no correlation with these factors in normal-weight healthy subjects (Figure 2B and Figure S4). An increase of the body mass index, serum insulin level, and CRP level is associated with the development of type 2 diabetes and atherosclerosis (Eckel et al., 2005; Mokdad et al., 2003). Indeed, serum Angptl2 was also significantly increased in patients with type 2 diabetes or coronary artery disease (Figure 2C). In 935 consecutive persons aged 27–84 years who underwent a medical checkup and gave informed consent for measurement of serum Angptl2 at the Japanese Red Cross Kumamoto Health Care Center, the Angptl2 level was positively correlated with the body mass index, abdominal circumference, and serum CRP level (Figure S5). In patients with type 2 diabetes, Angptl2 was positively correlated with the visceral fat area, homeostasis model assessment of insulin resistance (HOMA-R) index (Matthews et al., 1985), and serum CRP level, but not with the subcutaneous fat area. Angptl2 level was inversely correlated with the insulin sensitivity index (M value), as assessed

by the hyperinsulinemic euglycemic clamp test (DeFronzo et al., 1979) (Figure 2D).

These observations led us to ask whether improvement of systemic insulin resistance or inflammation would influence the circulating level of Angptl2. We observed a significant decrease of the plasma Angptl2 level in 27 obese diabetic men following treatment with pioglitazone at 30 mg/day for 3 months (Figure 2E). The percent decrease of the plasma Angptl2 level was correlated with the percent decrease of the visceral fat area, HOMA-R index, and serum CRP level (Figure 2F). These results suggested that visceral fat was likely to be the main source of circulating Angptl2, the concentration of which was significantly correlated with systemic insulin resistance and inflammation.

Angptl2 Activates Migration and Inflammatory Changes of Endothelial Cells and Monocytes/Macrophages via Integrins

Since the vasculature has an important role in tissue inflammation (Jackson et al., 1997), we examined the effect of Angptl2 on endothelial cells. First, we found a dose-dependent increase of cell adhesion when human umbilical vein endothelial cells (HUVECs) and human arterial endothelial cells (HAECs), which express several integrins on their surfaces, were plated on Angptl2-coated plates (Figures 3A and S6). We next analyzed cell adhesion in the presence of a series of function-blocking antibodies for specific integrins. A neutralizing antibody for integrin $\alpha5\beta1$ inhibited endothelial cell adhesion to Angptl2-coated plates, as did RGD peptide, which blocks RGD-dependent integrins (Figure 3B), suggesting that Angptl2-induced endothelial cell adhesion was an $\alpha5\beta1$-dependent process, although the involvement of untested integrins could not be excluded. Integrin $\alpha5\beta1$ activates NF-κB in endothelial cells (Klein et al., 2002). Consistently, there was increased translocation of NF-κB to the nucleus and degradation of IκB in HUVECs stimulated with recombinant human Angptl2 protein (Figures 3C and 3D).

Angptl2 also promoted the migration of HUVECs and HAECs through a microchemotaxis membrane (Figure 3E). Time-lapse imaging of HUVECs or HAECs cultures revealed that protrusion of lamellipodia and membrane ruffling were rapidly induced following the addition of Angptl2 (Movies S1 and S2). Since Rac1, a small Rho-GTPase, plays a pivotal role in the protrusion of lamellipodia, membrane ruffling, and cell migration (Bar-Sagi and Hall, 2000; Fryer and Field, 2005), we investigated whether Rac1 was activated in HAECs and HUVECs by performing a pull-down assay. Activation of Rac1 was detected in both Angptl2-stimulated HUVECs and HAECs (Figure 3F). In viable Angptl2-stimulated HUVECs, a single-molecule probe was used to determine Rac1 activity, showing that it was diffusely activated at the plasma membrane, with this activation being followed by protrusion of lamellipodia and membrane ruffling (Figure 3G and Movie S3). Moreover, Angptl2 no longer stimulated the protrusion of lamellipodia and membrane ruffling in HUVECs transfected with a dominant-negative Rac1 mutant expressing red fluorescent protein (RacN17-IRES-RFP) (Figure 3H and Movie S4). These findings suggest that Angptl2-stimulated lamellipodia formation and membrane ruffling in endothelial cells were both mediated by activation of Rac1. Next, we investigated whether Angptl2 could induce in vivo chemotaxis of endothelial cells in a mouse cornea assay. Implanted pellets containing Angptl2 markedly induced neovascularization in the mouse cornea, whereas pellets containing PBS alone did not (Figure 3I). Monocytes/macrophages express several integrin receptors that are responsible for adhesion, migration, and extravasation into the peripheral tissues (Friedl and Weigelin, 2008; Rose et al., 2007). We found that the THP-1 human monocytic cell line expressed integrins $\alpha4$, $\beta1$, $\beta2$, and $\alpha5\beta1$ (Figure 3J). THP-1 cells adhered to Angptl2-coated plates in a dose-dependent manner (Figure 3K). FACS analysis revealed that Angptl2 bound to THP-1; this binding was completely inhibited by neutralizing antibodies for integrins $\alpha4$ or $\beta2$ and was partially blocked by antibodies for integrin $\alpha5\beta1$ or $\beta1$ (Figure 3L). Angptl2 also promoted transmigration by THP-1 cells and primary human monocytes (Figure 3M and Figure S7).

Constitutive Angptl2 Activation Induces Local Inflammation in Mouse Skin Tissue

To further investigate the role of Angptl2 in the inflammatory process, we generated transgenic mice expressing Angptl2 driven by the keratinocyte-specific promoter K14 (K14-Angptl2) and therefore constitutively expressing Angptl2 in the epidermis (Figures S8A and S8B). The ears, snouts, and eyelids of K14-Angptl2 mice were redder than those of controls. The tails of K14-Angptl2 mice were not only reddish but also swollen and showed loss at the tips (Figure 4A), indicating local inflammation. Lectin staining showed an increase of adherent leukocytes, a common feature of inflammatory vasculature (McDonald, 1994), in enlarged vessels of the skin tissue specimens from K14-Angptl2 mice (Figure 4B), while there was no difference of vessel length between the genotypes (Figure S8C). The vessels of K14-Angptl2 mice were significantly more permeable than the vessels of wild-type controls after inflammation was induced by topical application of mustard oil, a potent proinflammatory agent (Figure 4C). As expected, even before mustard oil application, lumens of $CD31^+LYVE\text{-}1^+$ lymphatics were enlarged in the skin of K14-Angptl2 mice, while such changes were not observed in controls (Figure 4D), suggesting that increased drainage via lymphatics was compensating for the excessive leakiness of Angptl2-stimulated vessels in the dermis. These findings indicate that Angptl2 induces inflammatory vascular remodeling rather than angiogenesis.

Reduction of Adiposity and Obesity-Related Adipose Tissue Inflammation in *Angptl2*$^{-/-}$ Mice

Next, we investigated the pathophysiological role of Angptl2 by generating Angptl2 knockout (*Angptl2*$^{-/-}$) mice (Figure S9). *Angptl2*$^{-/-}$ mice were born alive following Mendelian inheritance and appeared to be grossly normal. Interestingly, when fed normal chow, *Angptl2*$^{-/-}$ mice weighed slightly less (Figure S10A) and had a lower body fat mass estimated by computed tomography (CT) (Figures S10B and S10C) than heterozygotes or wild-type mice fed the same normal diet, although there was no significant difference of daily food intake or energy expenditure between the groups (Figures S10D and S10E). In addition, *Angptl2*$^{-/-}$ mice showed slightly, but significant, better glucose tolerance and insulin sensitivity (Figures S10F and S10G). Next, we fed 8-week-old mice a high-fat diet containing 32% (wt/wt) fat to stimulate weight gain. After

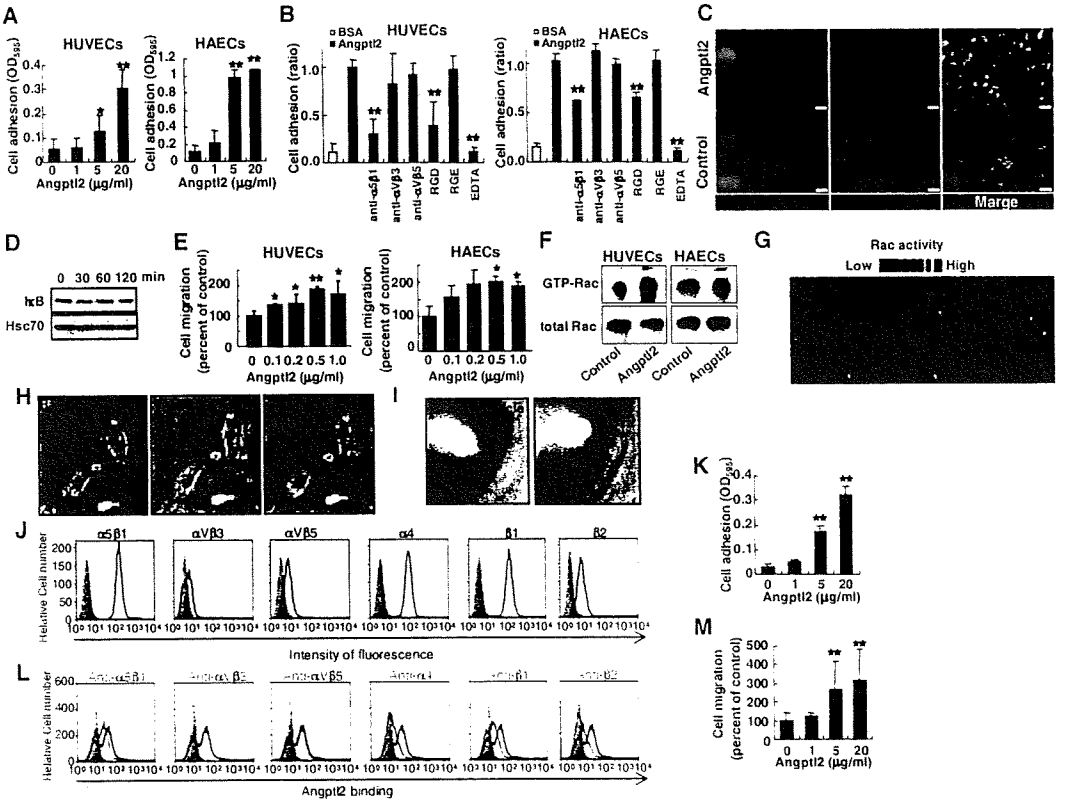

Figure 3. Angptl2 Activates Endothelial Cells and Monocytes

(A) Adhesion of HUVECs or HAECs to culture dishes coated with various concentrations of recombinant human Angptl2 (n = 3).

(B) HUVECs or HAECs were preincubated with or without 25 μg/ml of blocking antibodies (anti-α5β1, anti-αvβ3, or anti-αvβ5) or RGD or RGE peptides (300 μM), and cell adhesion was assessed (n = 3). As a negative control, cell adhesion was assayed in the presence of 10 μM EDTA, which inhibits integrin binding.

(C) Nuclear translocation of NF-κB subunit p65 in HUVECs at 2 hr after Angptl2 stimulation. Nuclei were counterstained with 4′,6′-diamidino-2-phenylindole (DAPI). Scale bar, 20 μm.

(D) Representative western blots of IκB and Hsc70 protein (internal control) in HUVECs at the indicated times after Angptl2 stimulation.

(E) Migration of HUVECs or HAECs in response to Angptl2 (n = 4).

(F) HUVECs or HAECs were cultured with Angptl2 for 30 min and then subjected to the pull-down assay using GST-PAK-CRIB followed by western blotting with anti-Rac1 antibody. Representative images are shown.

(G) HUVECs expressing Raichu-Rac1 (a probe for active Rac1) at the indicated times (min) after Angptl2 stimulation. Arrows indicate nascent and retracting lamellipodia. Ratio ranges are shown on the right.

(H) HUVECs that were either untransfected or transfected with RacN17 (shown in red in the left panel and by red stars in the center and right panels) and stimulated with Angptl2 at time 0 and 15 min. Angptl2-stimulated membrane ruffling is observed in HUVECs without RacN17 (arrows).

(I) Macroscopic appearance of neovascularization in the mouse cornea. Pellets containing vehicle or Angptl2 (0.5 μg) were implanted into micropockets cut in the corneal stroma.

(J) Integrin expression by THP-1 cells. Typical profiles obtained by FACS analysis with the indicated anti-integrin antibodies (black line traces) or isotype-matched control IgG (filled gray traces).

(K) Adhesion of THP-1 cells to culture dishes coated with various concentrations of Angptl2 (n = 3).

(L) Inhibition of Angptl2 binding to THP-1 cells by integrin-neutralizing antibodies. THP-1 cells were preincubated with (red line traces) or without (blue line traces) the indicated anti-integrin neutralizing antibodies, and then incubated with FLAG-tagged Angptl2 followed by detection with FITC-conjugated anti-FLAG antibody. Negative controls (filled gray traces) had omission of Angptl2.

(M) Migration of THP-1 cells in response to Angptl2 (n = 7–9). Data are the mean ± SD. $^{*}p < 0.05$ and $^{**}p < 0.01$ compared with controls.

8 weeks of high-fat diet feeding, *Angptl2*$^{-/-}$ mice had a body weight 12% lower than that of wild-type mice (Figure 5A). The visceral and subcutaneous fat mass and total body fat percentage were moderately decreased in *Angptl2*$^{-/-}$ mice compared to wild-type mice (Figures 5B and 5C). Considerable accumulation of fat was seen in the liver and skeletal muscle of wild-type mice, whereas these changes were mild in *Angptl2*$^{-/-}$ mice (Figures 5D and 5E). Although there were no obvious differences of food intake or energy expenditure between the two groups, the respiratory quotient was significantly lower in the *Angptl2*$^{-/-}$ group (Figures S11A–S11C).

We next examined the expression of mRNAs for inflammatory cytokines (IL-6 and TNF-α), a chemokine (MCP-1), various macrophage markers (F4/80, CD68, CCR2, Mgl1, and Mgl2),

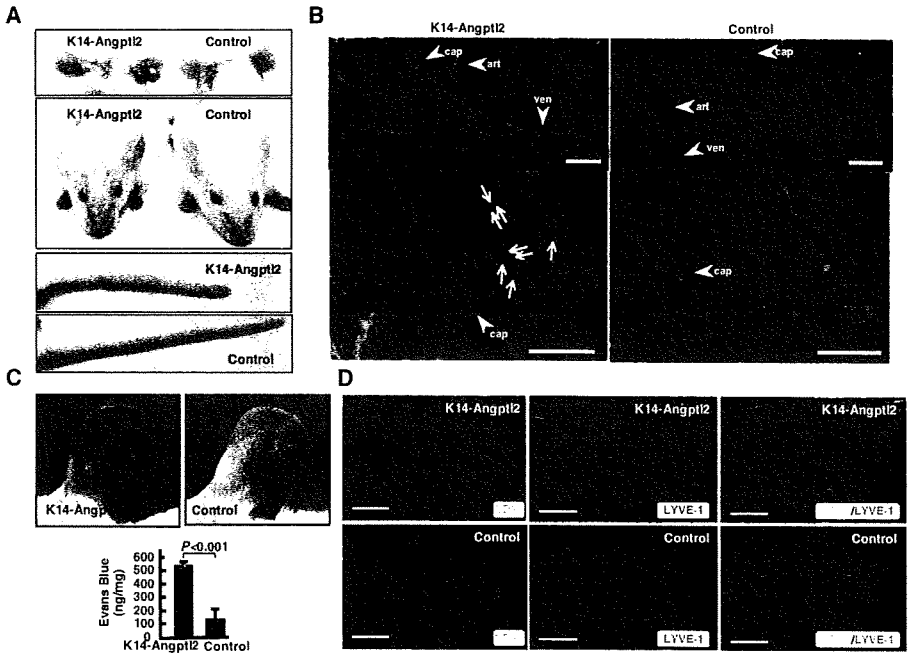

Figure 4. Sustained Angptl2 Overexpression Induces Vascular Inflammation

(A) Appearance of 6-month-old transgenic K14-Angptl2 and control mice.

(B) Ear skin blood vessels from transgenic and control mice. Arrows indicate adherent leukocytes on the walls of enlarged vessels from K14-Angptl2 mice. Arrowheads with art, ven, and cap in each panel indicate arteriole, venule, and capillary, respectively. Scale bar, 200 μm.

(C) Evans blue dye leakage into the skin of the ear following treatment with mustard oil as an inflammatory agent. Representative images and quantitative values are shown (mean ± SD, n = 7).

(D) Immunohistochemistry of ear skin from K14-Angptl2 and control mice with anti-CD31 and anti-LYVE-1 antibodies. Representative photographs are shown. Scale bar, 100 μm.

and insulin-sensitizing adipocytokines (adiponectin and leptin) in the adipose tissue of mice fed a high-fat diet. As shown in Figure 5F, adiponectin expression was increased, while TNF-α and general (F4/80, CD68) and inflammatory (CCR2) macrophage markers were all decreased in the adipose tissue of *Angptl2*$^{-/-}$ mice. However, the expression of residential macrophage markers (Mgl1 and Mgl2) remained unchanged. Furthermore, expression of F4/80 mRNA was positively correlated with the adipose tissue weight in controls, indicating that adiposity was significantly correlated with macrophage infiltration into adipose tissue. In contrast, there was no significant correlation between adipose tissue weight and macrophage infiltration in *Angptl2*$^{-/-}$ mice (Figure 5G), suggesting that this decrease of macrophage infiltration may be independent of reduced adiposity in *Angptl2*$^{-/-}$ mice. Furthermore, immunohistochemistry using the macrophage marker Mac2 revealed accumulation of Mac2-positive macrophages in crown-like structures within the adipose tissue of wild-type mice, while fewer Mac2-positive cells were observed in the adipose tissue of *Angptl2*$^{-/-}$ mice (Figure 5H). The high-fat diet caused impaired glucose tolerance and insulin resistance in controls, whereas *Angptl2*$^{-/-}$ mice showed better glucose tolerance and insulin sensitivity based on the results of intraperitoneal glucose and insulin tolerance tests (GTT and ITT, respectively) (Figures 5I and 5J). To explore which organ(s) contributed to the improved insulin sensitivity in *Angptl2*$^{-/-}$ mice, we next performed western blotting analysis of the insulin signaling pathway. Tyrosine phosphorylation of insulin receptor β and serine phosphorylation of Akt after insulin injection were significantly increased in both the liver and skeletal muscle of *Angptl2*$^{-/-}$ mice compared with wild-type mice (Figure 5K). To confirm these results, we performed hyperinsulinemic-euglycemic clamp experiments. Both glucose infusion rate and whole-body glucose disposal rate were significantly increased in *Angptl2*$^{-/-}$ mice, while clamp endogenous glucose production was significantly reduced. In addition, the percent decrease in endogenous glucose production from basal to clamp states was significantly higher in *Angptl2*$^{-/-}$ mice than in wild-type mice (Figure 5L). These results indicated that insulin sensitivity was improved in both the skeletal muscle and liver of *Angptl2*$^{-/-}$ mice fed a high-fat diet.

Angptl2 Promotes Local Inflammation in Adipose Tissue and Systemic Insulin Resistance

Finally, we determined whether sustained overexpression of Angptl2 in adipose tissue promoted systemic insulin resistance by generating transgenic mice that overexpressed Angptl2 in adipose tissue under the control of aP2, an adipose tissue-specific promoter (aP2-Angptl2) (Figure S12A). Based on the level of Angptl2 expression, we considered that line 5 was the most acceptable model for examining the pathological role of increased Angptl2 expression in obese mice (Figure S12B), so we performed subsequent analyses using line 5 and wild-type

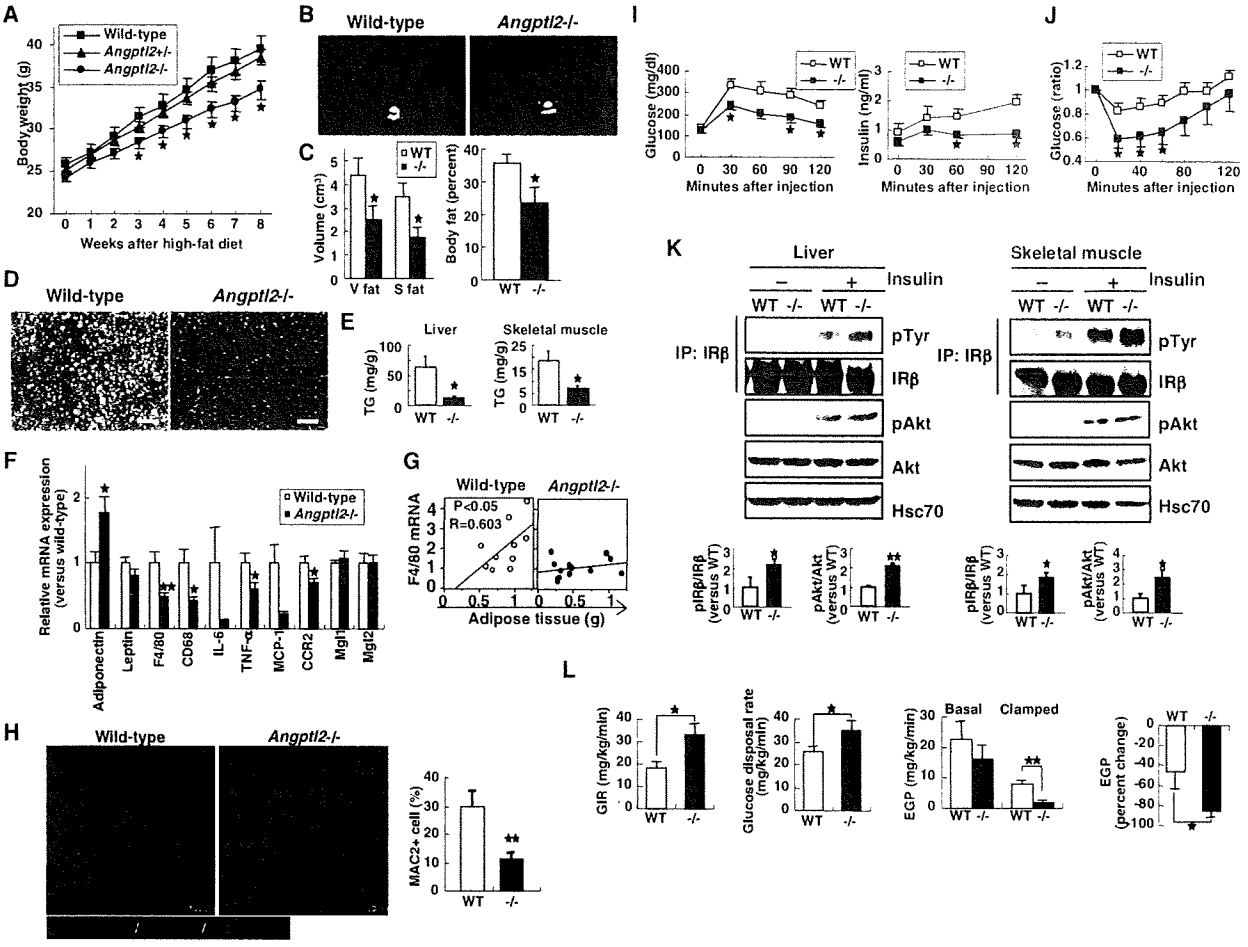

Figure 5. Deletion of Angptl2 Reduces Adipose Tissue Inflammation and Systemic Insulin Resistance in Dietary Obese Mice

Analyses of *Angptl2*$^{-/-}$ and wild-type mice fed a HFD for 8 weeks (A–L).

(A) Body weight of each genotype (n = 8–16 per group) at the indicated times (weeks) after initiation of a HFD.

(B and C) Representative CT findings (B) and quantitative comparison of the visceral (V) and subcutaneous (S) fat volume and total percent body fat (C) in *Angptl2*$^{-/-}$ mice and wild-type mice (n = 5–7 per group).

(D) HE-stained liver sections from *Angptl2*$^{-/-}$ and wild-type mice. Scale bar, 100 μm.

(E) Triglyceride (TG) content of liver and skeletal muscle from *Angptl2*$^{-/-}$ mice and wild-type mice (n = 6 per group).

(F) Quantitative RT-PCR of mRNAs encoding adipocytokines and macrophage markers in epididymal adipose tissue from *Angptl2*$^{-/-}$ and wild-type mice (n = 11–12 per group).

(G) Correlation between F4/80 mRNA expression and epididymal adipose tissue weight in *Angptl2*$^{-/-}$ mice and wild-type mice (n = 11–12 per group). Correlation coefficient (R) and probability (P) values are shown.

(H) Immunohistochemistry of adipose tissue using the macrophage marker MAC2 and adipocyte marker perillipin. Representative photographs and quantitative comparisons of MAC2-positive cells (n = 6 per group) are shown. Scale bar, 100 μm.

(I and J) Glucose (I) and insulin (J) tolerance tests in *Angptl2*$^{-/-}$ mice and wild-type mice (n = 5 and n = 10 per group, respectively).

(K) Insulin signaling in the liver and skeletal muscle of *Angptl2*$^{-/-}$ (KO) and wild-type (WT) mice. Representative western blots and quantitative data for the total and phosphorylated forms of insulin receptor β subunit (IRβ) and Akt are shown (n = 4 per group).

(L) Glucose infusion rate (GIR), glucose disposal rate, endogenous glucose production (EGP) during the basal and clamped states, and percent change in EGP between the states in *Angptl2*$^{-/-}$ (KO) and wild-type (WT) mice (n = 5–7 per group). Data are mean ± SEM, $^{*}p < 0.05$ and $^{**}p < 0.01$ compared with controls.

littermates as controls. There was no difference of weight gain between aP2-Angptl2 mice and control wild-type mice fed a normal chow diet (Figure 6A). However, immunohistochemistry using Mac2 revealed accumulation of macrophages in crown-like structures within the adipose tissue of aP2-Angptl2 mice, whereas fewer Mac2-positive cells were observed in wild-type mice (Figure 6B). RT-PCR analysis revealed that inflammatory cytokines (IL-6, TNF-α, and IL-1β) and general (CD68) and inflammatory (CCR2) macrophage markers were increased in the adipose tissue of aP2-Angptl2 mice, while adiponectin and leptin were unchanged (Figure 6C). Lectin staining showed an increase of adherent leukocytes in vessels within the adipose tissue of aP2-Angptl2 mice, while few leukocytes were detected in the vessels of wild-type mice (Figure 6D). There was no

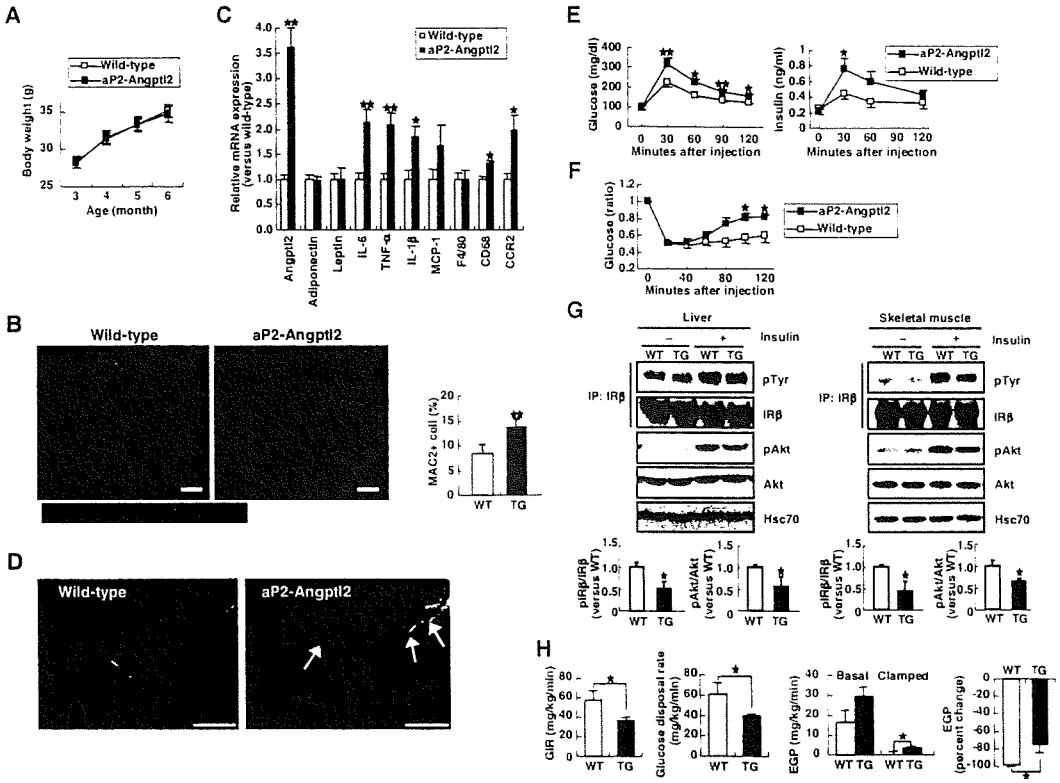

Figure 6. Angptl2 in Adipose Tissue Induces Local Inflammation and Systemic Insulin Resistance

Analyses of aP2-Angptl2 and wild-type mice at 16 weeks of age (B–H).

(A) Body weight of aP2-Angptl2 and wild-type mice (n = 14–16 per group) at the indicated ages (months).

(B) Immunohistochemistry of adipose tissue using the macrophage marker MAC2 and adipocyte marker perillipin. Representative photograghs and quantitative comparison of MAC2-positive cells are shown (n = 6 per group). Scale bar, 50 μm.

(C) Quantitative RT-PCR of mRNAs encoding adipocytokines and macrophage markers in epididymal adipose tissue from aP2-Angptl2 mice and wild-type mice (n = 6 per group).

(D) Blood vessels in epidydimal adipose tissue from aP2-Angptl2 and wild-type mice. Arrows indicate adherent leukocytes on the walls of enlarged vessels in aP2-Angptl2 mice. Scale bar, 25 μm.

(E and F) Glucose (E) and insulin (F) tolerance tests in aP2-Angptl2 mice and wild-type mice (n = 10–12 per group).

(G) Insulin signaling in the liver and skeletal muscle of aP2-Angptl2 (TG) and wild-type (WT) mice. Representative western blots and quantitative data for the total and phosphorylated forms of insulin receptor β subunit (IRβ) and Akt are shown (n = 4 per group).

(H) Glucose infusion rate (GIR), glucose disposal rate, endogenous glucose production (EGP) during the basal and clamped states, and percent change in EGP between the states in aP2-Angptl2 (TG) and wild-type (WT) mice (n = 7 per group). Data are mean ± SEM, $^{*}p < 0.05$ and $^{**}p < 0.01$ compared with controls.

difference of blood vessel density between aP2-Angptl2 mice and control mice (Figure S12C). aP2-Angptl2 mice showed glucose intolerance and insulin resistance in the GTT and ITT, respectively (Figures 6E and 6F). Insulin signaling was diminished in both liver and skeletal muscle of aP2-Angptl2 mice compared with wild-type mice (Figure 6G). The hyperinsulinemic-euglycemic clamp tests also revealed insulin resistance in both skeletal muscle and liver of aP2-Angptl2 mice, since the glucose infusion rate, whole-body glucose disposal rate, and percent change of endogenous glucose production between basal and clamp states were reduced in aP2-Angptl2 mice compared with wild-type mice, while hepatic glucose production during the clamp period was increased in aP2-Angptl2 mice compared with control mice (Figure 6H). These results indicated the presence of insulin resistance in both the skeletal muscle and liver of nonobese aP2-Angptl2 mice.

DISCUSSION

We demonstrated that Angptl2, a member of the Angptl family, is a key mediator of chronic adipose tissue inflammation and obesity-related systemic insulin resistance.

Here we showed that Angptl2 is an adipocyte-derived inflammatory mediator, with increased expression at both the mRNA and protein levels in obesity. Hypoxia and ER stress, which are enhanced in obese adipose tissue (Hosogai et al., 2007; Nishimura et al., 2008; Schenk et al., 2008; Ye, 2009), both increased Angptl2 expression or secretion in adipocytes. Various changes of the microenvironment observed in the adipose tissue of obese animals, such as inflammation and hypoxia, could also promote ER stress (Schenk et al., 2008). Therefore, Angptl2 production by adipocyte should be increased by hypoxia and ER stress in obesity.

It is noteworthy that the circulating Angptl2 level was positively correlated with obesity-related metabolic changes. The difference of circulating Angptl2 protein levels between Angptl2 Tg mice and wild-type mice was only 1.5-fold, but tissue Angptl2 levels showed a 3- to 5-fold difference (data not shown). Therefore, the modest difference of circulating Angptl2 levels in humans may reflect a larger alteration of adipose tissue Angptl2 expression, which could promote inflammation of adipose tissue, resulting in systemic insulin resistance. We also do not exclude the possibility that there is a direct inhibitory effect of circulating Angptl2 on insulin sensitivity in other peripheral tissues, such as skeletal muscle or the liver, because glucose clamp studies and western blotting analysis of insulin signaling revealed that both skeletal muscle and liver were target organs for Angptl2-related insulin resistance in mice. Other Angptl family molecules function in an endocrine manner to regulate lipids, glucose, and energy metabolism (Hato et al., 2008; Oike et al., 2005a, 2005b), so further studies are needed to clarify whether Angptl2 might also act in an endocrine manner.

Angptl2 contains an N-terminal coiled-coil domain and a C-terminal fibrinogen-like domain. The coiled-coil domain is required for oligomerization, which is necessary for its maximum activity, while the fibrinogen-like domain shares high homology with the analogous domain of fibrinogen. Fibrinogen acts as a ligand of the receptors for integrins such as $\alpha v\beta 3$, $\alpha 5\beta 1$, and $\alpha M\beta 2$ (Herrick et al., 1999; Mosesson, 2005), which are heterodimeric transmembrane glycoproteins that mediate cell-extracellular matrix and cell-cell adhesion (Hynes, 2002). Angptl3 was reported to promote angiogenesis through integrin $\alpha v\beta 3$ (Camenisch et al., 2002). In this study, we found that Angptl2 acted on endothelial cells through integrin $\alpha 5\beta 1$ and influenced monocytes/macrophages through integrins $\alpha 4$ or $\beta 2$. Several reports have indicated that integrin $\alpha 5\beta 1$ signaling activates Rac1 in endothelial cells (Dormond et al., 2001; Mettouchi et al., 2001), in agreement with our finding that Angptl2 promotes Rac1 activation in endothelial cells. We also found that Angptl2 induced the chemotaxis of endothelial cells by in vitro time-lapse imaging analysis and an in vivo mouse cornea neovascularization assay. In contrast, constitutive overexpression of Angptl2 in mouse skin or adipose tissue induced pathological vascular inflammation but did not increase vascularization or ameliorate hypoxia in the adipose tissue of mice with dietary obesity (Figure S12D). The cornea is an avascular tissue and thus is isolated from circulating soluble bioactive mediators, whereas various angiogenesis-related factors exist in highly vascular tissues such as the skin and adipose tissue. Taken together, these findings indicate that Angptl2 may function differently in different tissues, but it promotes vascular inflammation rather than angiogenesis, at least in adipose tissue that develops in obese mice.

Potentially relevant to these findings, we observed that Angptl2 stimulated the nuclear translocation of NF-κB and degradation of IκB in cultured vascular endothelial cells, findings consistent with a previous report that integrin $\alpha 5\beta 1$ signaling activates NF-κB-dependent expression of genes that are important for inflammation (Klein et al., 2002). There have been several other reports that Rac1 activates NF-κB (Perona et al., 1997; Sulciner et al., 1996), which is also consistent with our findings. An important aspect of inflammation is the recruitment of immune cells to affected tissues (Luster et al., 2005). This process requires adhesion of the immune cells to endothelial cells, allowing extravasation into the interstitium, followed by adhesion of immune cells to the extracellular matrix that enables migration toward the site of inflammation. In this regard, Angptl2 not only activated NF-κB in endothelial cells, which could induce expression of adhesion molecules (such as ICAM, VCAM, and selectin) and thus facilitate adhesion of immune cells to endothelial cells, but also promoted the migration of monocytes. Immune cells express integrins $\alpha 4$ or $\beta 2$, as well as $\alpha 5\beta 1$, which mediate cell adhesion, migration, activation, and production of proinflammatory cytokines through activation of NF-κB (Hynes, 2002; Rose et al., 2007; Roman et al., 2004; Graves and Roman, 1996), suggesting that Angptl2 may activate monocytes via such integrins. It remains to be clarified whether only Angptl2 among the Angptl family shows a stimulatory effect on adipose tissue inflammation, because some other members of this family bind to integrins (Camenisch et al., 2002), and Angptl4 is also abundantly expressed in adipose tissue. The skin tissue of K14-Angptl4 mice showed no inflammatory changes (Ito et al., 2003), unlike that of K14-Angptl2 mice. Moreover, there was no correlation between the serum Angptl4 concentration and Angptl2-related metabolic factors. These findings suggest that the effects of Angptl4 on endothelial cells and/or immune cells are different from those of Angptl2.

In this study, we demonstrated that Angptl2 deletion not only ameliorated inflammation in adipose tissue but also improved systemic insulin resistance in mice with dietary obesity, although it did not completely normalize their insulin sensitivity to the level seen in mice fed a normal chow diet (Figure S11I). The restoration of insulin sensitivity related to Angptl2 deletion may be attributable to the difference of body fat accumulation between the two genotypes. Since adipose tissue volume was not correlated with macrophage infiltration in *Angptl2*$^{-/-}$ mice, some mechanism other than the difference of adiposity may also have contributed to reducing adipose tissue inflammation in *Angptl2*$^{-/-}$ mice. Actually, constitutive Angptl2 overexpression in adipose tissue induced both local inflammation and systemic insulin resistance in nonobese mice. Since adipose tissue inflammation can be a cause of systemic insulin resistance via the secretion of several inflammatory factors (Apovian et al., 2008; Neels and Olefsky, 2006; Schenk et al., 2008), it is suggested that Angptl2 probably influenced systemic insulin sensitivity by exacerbating adipose tissue inflammation (Figure S13).

Although a reduction of adipose tissue inflammation could well be the main reason for improvement of insulin sensitivity in *Angptl2*$^{-/-}$ mice, some other possible mechanisms remain. Adiponectin can potentially increase insulin sensitivity, and the adiponectin level is usually decreased in obesity (Kadowaki and Yamauchi, 2005). However, there was no difference of circulating adiponecin levels between *Angptl2*$^{-/-}$ and control mice (Figure S11H). On the other hand, *Angptl2*$^{-/-}$ mice had a reduced triglyceride content in both skeletal muscle and liver, which could improve insulin sensitivity in these two organs (Schenk et al., 2008).

Angptl2$^{-/-}$ mice showed reduced body fat and tissue triglyceride accumulation when fed a high-fat diet, although there was no obvious difference of daily food intake and energy expenditure estimated from the O_2 consumption rate. Interestingly, the respiratory quotient of *Angptl2*$^{-/-}$ mice was significantly lower

than that of wild-type mice, suggesting that *Angptl2*$^{-/-}$ mice were more likely to use lipids than carbohydrates for oxidation to create energy. There was also a trend of increased expression of lipid oxidation genes in the skeletal muscle of *Angptl2*$^{-/-}$ mice and increased UCP1 expression in brown adipose tissue (Figures S11D and S11F), which may account for the lower respiratory quotient, decreased triglyceride content of skeletal muscle, and decrease of whole-body fat in *Angptl2*$^{-/-}$ mice. On the other hand, the hepatic expression of lipogenic genes (SREBP-1c, FAS, and SCD1) was significantly decreased in *Angptl2*$^{-/-}$ mice (Figure S11G), which explains the decreased triglyceride content in the liver of these mice, although further studies will be needed to clarify the molecular mechanisms involved.

In summary, this study provided evidence that Angptl2 plays a key role in inflammation of adipose tissue via inflammatory vascular remodeling and recruitment of macrophages into adipose tissue. These findings suggest that Angptl2 may be an important part of the mechanism underlying adipose tissue inflammation that is involved in the pathogenesis of systemic insulin resistance related to obesity. The present findings should also lead to new treatment strategies for obesity and related insulin resistance.

EXPERIMENTAL PROCEDURES

Materials and additional methods are available in the Supplemental Experimental Procedures.

Animal Study

All experimental protocols were approved by the Ethics Review Committee for Animal Experimentation of Kumamoto University. Only male mice were used for the experiments. For the metabolic analyses, mice at 8 weeks of age were fed either a normal diet (CE-2; CLEA, Japan) or a high-fat diet (HFD-32; CLEA) for a period of 8 weeks. During the analyses, mice continued to feed on the same diet.

Human Studies

White adipose tissue samples were obtained from the intact adipose tissue surrounding the tumor resected from a patient with pancreatic carcinoma. Samples were fixed in 4% paraformaldehyde for 24 hr and embedded in paraffin. Sections 5 μm thick were cut and stained with an anti-Angptl2 polyclonal antibody (#383). Nuclei were counterstained with hematoxylin. A total of 98 volunteers working at Kumamoto University were enrolled in the study as the healthy group (persons with obesity [body mass index > 30] or diabetes were excluded). Blood samples were collected, and the plasma glucose, insulin, and CRP levels were measured. A total of 89 patients with type 2 diabetes were enrolled as the DM group. Their abdominal fat content was evaluated by magnetic resonance imaging. The HOMA-R index was calculated as the product of fasting plasma insulin (μU/ml) and fasting plasma glucose (mg/dl) divided by 405 (Matthews et al., 1985). The euglycemic-hyperinsulinemic clamp test was carried out according to a protocol described elsewhere (DeFronzo et al., 1979). A total of 109 patients with coronary artery disease (diagnosed by coronary angiography) were enrolled as the CAD group, and blood samples were collected. Twenty-seven obese diabetic men who had not previously received any antidiabetic agents, antihypertensive agents, or lipid-lowering drugs were treated with pioglitazone at a dose of 30 mg/day for 3 months. Before and after treatment, the abdominal fat content was evaluated by CT scanning, and fasting blood samples were collected to measure the levels of glucose, insulin, and CRP. Blood samples were also collected from 935 consecutive volunteers aged 27–84 years, who underwent medical checkups at the Japanese Red Cross Kumamoto Health Care Center. Serum or plasma levels of Angptl2 and Angptl4 were measured by ELISA. This study was approved by the Ethics Committees of Kumamoto University (healthy and CAD groups), Kobe University (DM group), Ryukyu University (pioglitazone study), and the Japanese Red Cross Kumamoto Health Care Center. Written informed consent was obtained from each subject.

SUPPLEMENTAL DATA

Supplemental Data include Supplemental Experimental Procedures, Supplemental References, 13 figures, one table, and four movies and can be found with this article online at http://www.cell.com/cell-metabolism/supplemental/S1550-4131(09)00232-0.

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Incidence and Outcomes of Out-of-Hospital Cardiac Arrest in the Eastern Part of Yamaguchi Prefecture

Teruo SHIRAKI,[1] MD, Kazuhiro OSAWA,[1] MD, Hideyuki SUZUKI,[1] MD, Masatoki YOSHIDA,[1] MD, Natsuki TAKAHASHI,[1] MD, Kazufumi TAKEUCHI,[1] MD, Machiko TANAKAYA,[1] MD, Kunihisa KOHNO,[1] MD, *and* Daiji SAITO,[1] MD

SUMMARY

The aim of the present study was to evaluate the factors related to poor prognosis of out-of-hospital cardiac arrest patients in one local area of Japan. From May 1, 2002 to April 30, 2008, a total of 442 patients with cardiopulmonary arrest were transferred for resuscitation to the National Hospital Organization, Iwakuni Clinical Center. Of 325 patients with cardiopulmonary arrest of cardiac etiology, 126 patients were witnessed by a bystander. However, only 37 received bystander cardiopulmonary resuscitation, 13 had shockable cardiac rhythm, 3 survived 1 month, and 2 had a good neurological discharge. Multivariate analysis of overall cardiac arrest showed that 1-month survival and neurologically favorable discharge were associated with bystander cardiopulmonary resuscitation (P = 0.049 and 0.013) and initial shockable cardiac rhythm (P = 0.001 and 0.007). In this region, the survival rate for patients with cardiopulmonary arrest was lower than that reported in other areas, probably because fewer patients received bystander CPR or had shockable cardiac rhythm. This may result from CPR being less popularized in this region than in other areas, suggesting that raising the awareness of CPR would improve the survival rate. (Int Heart J 2009; 50: 489-500)

Key words: Cardiopulmonary resuscitation, Ventricular fibrillation, Out-of-hospital cardiac arrest

INTRINSIC out-of-hospital cardiac arrest is associated primarily with cardiac disease, especially fatal arrhythmia represented by ventricular fibrillation (VF). Therefore, early defibrillation is an effective therapy for out-of-hospital cardiac arrest. In Japan, defibrillation by an emergency crew, previously allowed only under the direction of physicians, is now permitted to be performed under com-

From the [1] Department of Cardiology, National Hospital Organization, Iwakuni Clinical Center, Yamaguchi, Japan.
Address for correspondence: Teruo Shiraki, MD. Department of Cardiology, National Hospital Organization, Iwakuni Clinical Center, 2-5-1 Kuroisocho, Iwakuni, Yamaguchi 740-8510, Japan.
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prehensive direction, along with administration of medication. An increasing number of public facilities have been equipped with automated external defibrillators (AED), and cardiopulmonary resuscitation education, including public access defibrillation for local citizens, is being promoted. Despite these efforts, however, the prognosis of out-of-hospital cardiac arrest is very poor. Thus, the aim of this study was to investigate the incidence and outcomes of out-of-hospital cardiac arrest to evaluate the factors associated with poor prognosis in one local area of Japan.

METHODS

Emergency medical service system in the Iwakuni Medical Management Area: The Iwakuni Medical Management Area (IMM area), determined by the Yamaguchi Prefectural Government to be one of 8 medical management areas in the prefecture, has approximately 142,000 residents in an 883-km^2 area. There are 11 fire stations with a corresponding number of emergency dispatch centers in this area. The emergency medical service system (EMS) is operated by the local fire stations and activated by phoning 119. Highly trained prehospital emergency care providers are on duty 24 hours a day. During most of this study, emergency lifesaving technicians were allowed only to insert an intravenous line including epinephrine administration and an adjunct airway, and to use a semiautomated defibrillator under the direction of an online physician or an AED along with medication for out-of-hospital cardiac arrest patients. No medications, including epinephrine, were allowed to be administered by the provider. Three emergency care providers including 1 emergency lifesaving technician were in an ambulance. The number of emergency lifesaving technicians in the Iwakuni EMS was 13.5% of prehospital emergency care providers, which was almost equal to the average for Yamaguchi prefecture (14.4%).

Emergency room staff in the Iwakuni Clinical Center: The emergency medical team at the National Hospital Organization, Iwakuni Clinical Center (NHO-ICC) is staffed 24 hours a day by 4 physicians (internist, surgeon, anesthesiologist, cardiologist) and 3 nurses. They attempted to determine the etiology of the sudden cardiac arrests based on laboratory, radiographic, and any other examinations available 24 hours a day, including autopsy, during treatment.

Study design and participants: A total of 708 patients experienced out-of-hospital cardiac arrest at the time of ambulance arrival during the period of May 1, 2002 through April 30, 2008 in the IMM area. Among them, 215 patients were transferred to other hospitals or judged to be dead at the scene, and 493 were transferred for cardiopulmonary resuscitation (CPR) to the NHO-ICC. Excluding 51 patients for whom death was confirmed on arrival at the emergency room,

we enrolled 442 patients in our observational study.

Measures: Patient information was collected with a data form that included all core data recommended in the Utstein-style reporting guidelines for cardiac arrest[1]; age, sex, witnessed, initial cardiac rhythm, bystander CPR, response intervals, treatment, 24-hour and 1-month survival, and neurologically favorable discharge defined as category 1 or 2 based on the cerebral performance category (CPC) score.

Cardiac arrest was presumed to be of cardiac origin unless its cause fell into a specific subcategory including sudden infant death syndrome (SIDS), drug overdose, suicide, drowning, hypoxia, exsanguination, cerebrovascular accident, subarachnoid hemorrhage, and trauma, which were categorized as noncardiac etiologies. All data of arrests with any etiology were summarized in the Utstein Style Template. It is a widespread, standardized, uniform reporting system which can be used for the comparison of data regarding cardiac arrest with other areas of Japan or other countries. Initial cardiac rhythm was recorded and diagnosed by EMS personnel using off-line ECG monitors and/or automated defibrillators, and was confirmed by a physician. All patients who returned spontaneous circulation were admitted immediately to the intensive care unit (ICU). The times of collapse and initiation of bystander CPR were recorded by EMS interviews with the bystander, before leaving the scene. The data were collected under the conditions of patient anonymity and impossible connections, according to the ethics guideline for epidemiological surveys published by the Japanese Ministry of Health, Labor and Welfare.

Statistical analysis: Data were evaluated with ANOVA or Student's *t*-test for numerical variables and the χ^2 test for categorical variables. Multivariable logistic regression analysis was applied to estimate the relationship among variables before arriving at the hospital and either at 24 hours or one month after arriving at the hospital, plus neurologically favorable discharge. Odds ratios and their 95% confidence intervals were calculated. Statistical analysis was performed using SAS software version 9.13 (SAS Institute Inc, Cary, NC, USA).

RESULTS

Incidence of out-of-hospital cardiac arrest: The mean population-based incidence of out-of-hospital cardiac arrest patients in the IMM-area was 83.0 per 100,000 person-years. A total of 442 patients, 70% of all patients who experienced out-of-hospital cardiac arrest in the IMM area, were transferred to the NHO-ICC during the 6-year study period. Patient characteristics are described in Table I. The year-to-year incidence varied over a relatively narrow range: 70 to 80 patients with cardiac arrest visited the NHO-ICC annually. Forty-three percent of

Table I. Patient Characteristics

Sex (male : female)	243 : 199
Mean age (years)	71.3 ± 17.7
Interval (minutes)	
Collapse and call for ambulance	6.4 ± 6.6*
Collapse and bystander CPR	8.1 ± 11.4**
Call response interval	7.1 ± 3.9
Response-hospital interval	21.7 ± 12.2

*Time interval between collapse and call for ambulance was calculated using data from 191 patients whose collapse was witnessed.
**Time interval between collapse and bystander CPR was calculated using data from 39 witnessed patients with bystander CPR.
CPR indicates cardiopulmonary resuscitation.

Table II. Overview of Cardiac Arrest Presumed to Be of Cardiac Origin

	Cardiac arrest presumed to be of cardiac origin (n = 325)															
	Arrest witnessed by bystander (n = 126)						Arrest witnessed by EMS (n = 10)						Arrest not witnessed (n = 189)			
	Asystole		VF/VT		PEA		Asystole		VF/VT		PEA		Asystole		VF/VT		PEA	
Initial cardiac rhyrhm	86		13		27		4		2		4		160		6		23	
	yes	no	yes	no	yes	no	yes	no	yes	no	yes	no	yes	no	yes	no	yes	no
Bystander CPR	21	64	6	7	4	23							6	154	0	6	0	23
ROSC	8	15	4	2	0	10	2		1		3		2	19	0	1	0	6
Admission	8	15	4	2	0	10	2		1		3		2	19	0	1	0	6
Survival at 24 hours	3	5	3	3	0	7	1		0		2		1	11	0	1	0	4
Survival at 1 month	0	0	2	1	0	0	1		0		2		1	0	0	1	0	0
Neurologically favorable discharge	0	0	2	0	0	0	1		0		2		0	0	0	1	0	0

EMS indicates emergency medical system; VF, ventricular fibrillation; VT, ventricular tachycardia; PEA, pulseless electrical activity; CPR, cardiopulmonary resuscitation; and ROSC, return of spontaneous circulation.

overall patients were witnessed by a bystander and/or EMS personnel (Tables II and III).

Along with an increase in age, the incidence of cardiac arrest patients increased exponentially in both sexes. The incidence curve fitted well to the following equations: $y = 25.7e^{0.43x}$, $r = 0.956$ for males and $y = 7.66e^{0.55x}$, $r = 0.966$ for females.

Of 442 patients, 243 (55.0%) were male, and this sex difference for incidence was observed in every age group, even after correction by population for those less than 90 years of age (Figure 1).

Incidence along with month and season: Figure 2 demonstrates that cardiac arrest occurred most frequently in January and least frequently in August. When grouped into the four seasons (March, April and May for spring; June, July

Table III. Overview of Cardiac Arrest Presumed to Be of Noncardiac Origin

	Cardiac arrest presumed to be of noncardiac origin (n = 117)														
	Arrest witnessed by bystander (n = 54)						Arrest witnessed by EMS (n = 1)			Arrest not witnessed (n = 62)					
	Asystole		VF/VT		PEA		Asystole	VF/VT	PEA	Asystole		VF/VT		PEA	
Initial cardiac rhythm	33		5		16		0	0	1	55		3		4	
	yes	no	yes	no		no	yes no	yes no	yes no	yes	no	yes	no	yes	no
Bystander CPR	5	28	2	3	1	15				4	51	0	3	0	4
ROSC	2	2	1	1	1	12	0	0	0	1	10	0	1	0	2
Admission	2	2	1	1	1	12	0	0	0	1	10	0	1	0	2
Survival at 24 hours	2	1	1	0	1	11	0	0	0	0	7	0	1	0	2
Survival at 1 month	0	0	1	0	0	3	0	0	0	0	0	0	1	0	0
Neurologically favorable discharge	0	0	1	0	0	2	0	0	0	0	0	0	0	0	0

Abbreviations as in Tables I and II.

and August for summer; September, October and November for autumn; and December, January and February for winter), cardiac arrest occurred more frequently in winter than in the other 3 seasons ($P < 0.001$).

Etiology, witnessed, and bystander CPR: A total of 325 patients (73.5%) were suspected to have cardiogenic cardiac arrest, including 177 patients (40%) with evidence of cardiac disease and 148 patients (33.4%) with no known cause. Cardiac arrest in 24 patients (5.4%) was assumed to have a cerebrovascular accident etiology. Although more than 80% of incidents occurred in the subject's own home, only 191 cardiac arrests (43.2%) were witnessed. Public places, the workplace, nursing homes, or other long-term care facilities comprised the remaining locations. Bystander CPR was performed on 49 of 442 cardiac arrest patients, including 37 out of 325 cases of presumed cardiac origin (Tables II and III).

Time intervals and initial cardiac rhythm: As 251 collapses were not witnessed, the mean time interval between collapse and call for an ambulance was calculated using data from the remaining 191 patients for whom cardiac arrest was witnessed. The mean time interval between collapse to bystander CPR was calculated with data from 39 patients whose collapse was witnessed by a bystander who performed CPR. The mean time intervals from call for to arrival of an ambulance, and from call for an ambulance to arriving at the hospital were calculated with data from all 442 patients (Table I). It took 6.4 ± 6.6 minutes from collapse to the call for an ambulance. A long time interval was needed from collapse to bystander CPR (8.1 ± 11.4 minutes). The time intervals between call for and arrival of an ambulance at the scene and intervals between call for an ambulance and arrival at the hospital were 7.1 ± 3.9 and 21.7 ± 12.2 minutes, respectively. In all cases, initial ECGs recorded by EMS personnel showed pulseless

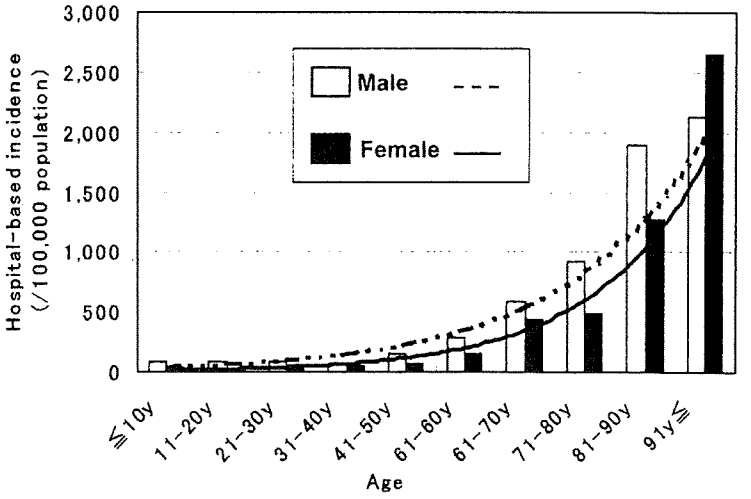

Figure 1. Incidence rate of out-of-hospital cardiac arrest patients transferred to the National Hospital Organization, Iwakuni Clinical Center (NHO-ICC) classified according to age and sex. Incidence rate increased exponentially with age for both sexes.
Men had a higher annual incidence rate than women in every age group younger than 90 years.

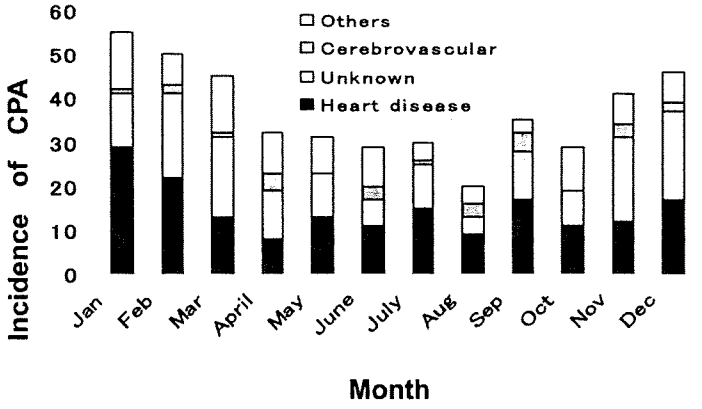

Figure 2. Monthly trend of out-of-hospital cardiac arrest at NHO-ICC.
Bars show the incidence of cardiac arrest, including definite heart disease, unknown origin, cerebrovascular disease, and other origins. This event occurred most frequently in January and least frequently in August.

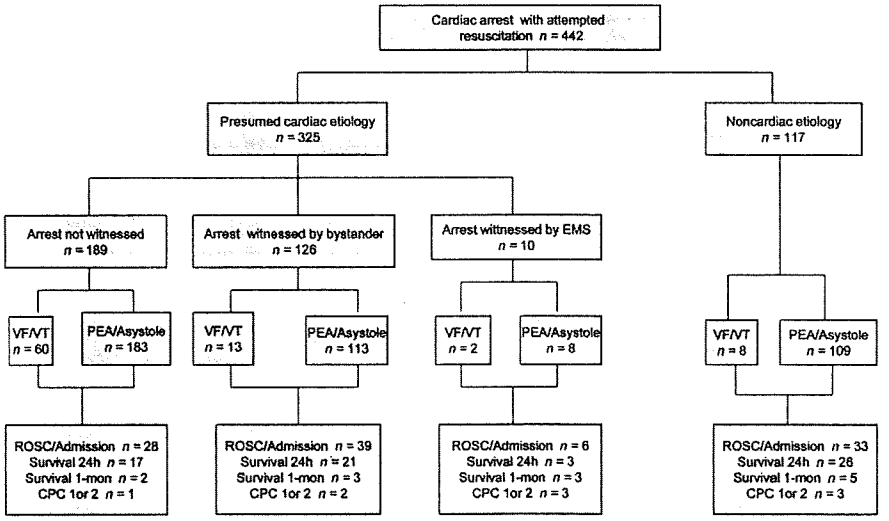

Figure 3. Overview of overall cases of out-of-hospital cardiac arrest with cardiac and noncardiac etiology based on an abridged Utstein type template.
VF indicates ventricular fibrillation; VT, ventricular tachycardia; PEA, pulseless electrical activity; ROSC, return of spontaneous circulation; and CPC, cerebral performance category.

electrical activity (PEA) in 75 patients, ventricular fibrillation (VF) in 29, and asystole in 338 (Figure 3). During the study period, no local citizen performed defibrillation with an AED. After the emergency crew arrived at the scene, 23 of 29 patients with VF as the presenting cardiac rhythm received defibrillation with a semiautomated defibrillator under the direction of physicians or with an AED under comprehensive direction.

Factors affecting prognosis: Of 442 out-of-hospital cardiac arrest patients, almost 85% died within 24 hours of arriving at the hospital, however, 15% of patients (67 patients) survived for more than 24 hours (Figure 3). Thirteen patients were alive at 1 month after admission, and are listed in Table IV. Nine patients (2.0%) were discharged from the hospital with a good neurological outcome (Table IV, patients 1 to 9); however, 4 patients (Table IV, patients 10 to 13) died due to respiratory infection and/or congestive heart failure without a significant improvement of their consciousness level. Limiting the data to cardiac arrest of presumed cardiac origin, the 24-hour and 1-month survival rates were 12.6% and 2.4%, respectively.

Variables entered into the logistic regression model were sex, age, etiology (cardiac versus noncardiac), initial cardiac rhythm (shockable versus nonshockable rhythm), witnessed status (witnessed versus nonwitnessed), CPR initiated by bystander (yes versus no) and intervals between call for and arrival of ambu-

Table IV. One-Month Survival

Case No.	Age	Sex	Etiology	Cardiac rhythm		Bystander	Time interval	
				Initial	At hospital	CPR	Call to scene	Call to hospital
1	80	M	heart	VF	VF	no	6 minute	25 minute
2	67	M	heart	PEA	VF	yes	5 minute	9 minute
3	66	M	heart	PEA	PEA	yes	3 minute	28 minute
4	67	F	heart	Asystole	VF	yes	3 minute	12 minute
5	65	F	heart	VF	VF	yes	4 minute	16 minute
6	52	M	unknown	VF	VF	yes	7 minute	24 minute
7	71	M	CVA	PEA	PEA	no	8 minute	49 minute
8	80	F	other	PEA	Asystole	no	7 minute	27 minute
9	74	F	other	VF	PEA	yes	5 minute	69 minute
10	62	M	heart	VF	VF	no	5 minute	15 minute
11	87	M	other	PEA	PEA	no	4 minute	14 minute
12	77	M	unknown	Asystole	Asystole	yes	8 minute	25 minute
13	55	F	unknown	VF	VF	no	6 minute	37 minute

Patients from No.1 to 9 discharged from hospital with favorable neurological outcome, and from No.10 to 13 were dead more than 1 month after admission.
CVA indicates cerebrovascular accident; VF, ventricular fibrillation; PEA, pulseless electrical activity; call to scene, call for until arrival of ambulance; and call to hospital, call for ambulance until patient arrival at hospital.

Table V. Prognositic Predictors for 24-Hour and 1-Month Survival and Neurologically Favorable Discharge

Variable	24-hour survival		1-month survival		Neurologically favorable discharge	
	Odds ratio (95%IC)	*P*	Odds ratio (95%IC)	*P*	Odds ratio (95%IC)	*P*
Sex (male versus female)	1.12 (0.62-2.00)	0.715	1.02 (0.97-1.67)	0.429	4.01 (0.66-24.59)	0.133
Age	1.01 (0.99-1.03)	0.383	5.28 (1.11-24.99)	0.036	1.03 (0.96-1.09)	0.445
Etiology (noncardiac versus cardiac)	2.66 (1.28-5.51)	0.008	5.47 (1.05-28.39)	0.043	6.60 (0.84-51.89)	0.073
Initial rhythm (shockable versus nonshockable)	3.14 (1.21-8.15)	0.018	15.77 (3.07-81.02)	0.001	29.40 (2.49-347.19)	0.007
Witnessed (yes versus no)	1.21 (0.66-2.25)	0.540	3.72 (0.66-21.15)	0.138	2.37 (0.20-28.40)	0.497
Bystander CPR (yes versus no)	1.64 (0.75-3.55)	0.214	4.37 (1.01-18.92)	0.049	12.97 (1.73-96.76)	0.013
Time interval						
Call for until arrival of ambulance	1.03 (0.95-1.11)	0.504	0.92 (0.77-1.12)	0.425	0.91 (0.72-1.14)	0.401
Call for ambulance until arrival at the hospital	0.98 (0.96-1.01)	0.182	0.98 (0.93-1.02)	0.317	0.99 (0.94-1.04)	0.609

CI indicates confidence interval. Other abbreviations as in Tables I and II.

lance, plus between call for ambulance and arrival at the NHO-ICC.

Table V shows the independent influence of 8 factors of importance for outcomes in CPR. Two variables, bystander CPR and initial shockable cardiac rhythm, were associated independently with an increased chance of survival at

all 3 observation points (24-hour and 1-month survival and neurologically favorable discharge). Noncardiac etiology was associated significantly with 24-hour and 1-month survival, while this variable was not a significant factor affecting discharge with good neurological outcome ($P = 0.073$). Male sex was associated with an increase in 1-month survival, but did not influence the chance of survival at 24 hours or neurologically favorable discharge.

Discussion

The results of this study demonstrated the overall characteristics and outcomes of out-of-hospital cardiac arrest patients transferred to our hospital. They indicate the importance of initial shockable cardiac rhythm and bystander CPR for a good prognosis. The NHO-ICC is the only hospital responsible for high-grade emergency medicine for 24 hours a day in the IMM area, so that approximately 70% of patients with out-of-hospital cardiac arrest are transferred to this center by ambulance for CPR. This means that the analyzed data in this study represent the present status of out-of-hospital cardiac arrest in this area. Therefore, this was a hospital-based study, but the results may be close to those of a community-based study.

The mean incidence of out-of-hospital cardiac arrest was high compared to that found in a study conducted in Osaka,[2] which reported an incidence of 63 per 100,000 person-years. The incidence at our hospital was higher in males than females in every age group except for those 90 years of age or over. The sex difference and exponential increase in incidence rate with increase in age agree with observations from studies conducted in Chicago[3] and Osaka.[4]

We also analyzed the survival rate at 24 hours after arriving at the hospital. Although we were not aware of other investigations that analyzed 24-hour survival, the survival rate could be an important variable for resuscitation of cardiac arrest patients. This variable provided information on whether improved hemodynamics, at least in the short-term, resulted in prolonging the patient's life. Better results for 24-hour survival might encourage us to undertake greater efforts for resuscitation. However, at 24 hours after arriving at this hospital, 15% of 442 cardiac arrest patients survived with successful resuscitation. When the survival rate was limited to patients with cardiac arrest of presumed cardiac origin, the rate decreased to 12.6%. The 1-month survival rate was 2.4% in patients with cardiogenic cardiac arrest witnessed by a bystander. It was 3.6% in patients with cardiogenic and noncardiogenic cardiac arrest witnessed by a bystander. The former was 4.5-5.1% in the studies conducted in Tokyo[5] and in Osaka,[2] the latter was 6.1% in the study conducted in Tokyo,[5] and both were markedly high compared to our study. This may be partly attributed to the patient profile as

it was performed on 31% in the central part of the prefecture.[9,12] We could not determine the reason why so many patients with cardiac arrest did not receive bystander CPR in the IMM area, similar to the western part of Yamaguchi prefecture. Currently, CPR training programs are provided not only in schools and driving schools, but also in public places under the direction of various organizations, such as the Fire Department and the Japanese Red Cross Society. In addition, the training program established by the American Heart Association (AHA), which has a long tradition and extensive experience in CPR training, has been introduced and is provided frequently in Japan. Since 2003, this hospital has provided CPR training program to local citizens, although the number of participants has not been large. In 2007, approximately 2,000 of 142,000 residents in the IMM area were provided basic life support training using an AED by the Fire Department, the proportion of which was similar to the average for all of Yamaguchi prefecture (21,600 of 1,465,000 residents). However, we suspected that the people in these training programs were younger people who lived in more urban areas. This was because bystander CPR was performed more often in public places, where younger providers tend to gather during the day, than in private residences (30% versus 10% in our study). Therefore, we suspected that people in the IMM area, especially in more rural areas, did not have as much chance to study cardiac resuscitation. In the IMM area, people over 65 years of age accounted for 26.1% of the total population (Population Survey in 2005). This rate is higher than the mean in Japan (20.1%) (Population Survey in 2005). Despite an increase in the availability of CPR training opportunities, the training of local elderly citizens is still not sufficient in this region, leaving bystander CPR unfamiliar to most. Moreover, overly complicated CPR with mouth-to-mouth rescue breathing is difficult to teach to the public, especially the elderly, in Japan. Thus, in addition to few chances to study the methods, elderly people are more likely to hesitate in applying resuscitation compared to younger people, even if they have knowledge of resuscitation methods.

In recent years, the usefulness of CPR with chest compression alone has been demonstrated primarily by Japanese studies, leading to the introduction of simplified CPR training.[2,5] The spread of simplified CPR is expected to reduce the resistance to CPR and thus increase its prevalence, eventually improving long-term prognoses for those having cardiac arrest events.

It is thought that current advanced cardiovascular life support (ACLS) may not affect long-term prognosis for out-of-hospital cardiac arrest.[13] In ACLS however, it has been demonstrated that mild hypothermia therapy following return of spontaneous circulation is associated with discharge with a neurologically favorable prognosis.[6,7] It may be important to introduce this therapy in our hospital as part of ACLS in order to improve the prognosis for out-of-hospital

cardiac arrest.

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