Decreased phospho-Akt signaling in a mouse model of total parenteral nutrition: a potential mechanism for the development of intestinal mucosal atrophy

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Feng Y, McDunn JE, Teitelbaum DH. Decreased phospho-Akt signaling in a mouse model of total parenteral nutrition: a potential mechanism for the development of intestinal mucosal atrophy. Am J Physiol Gastrointest Liver Physiol 298: G833–G841, 2010. First published March 18, 2010; doi:10.1152/ajpgi.00030.2010.—Total parenteral nutrition (TPN) leads to a decline in phosphatidylinositol 3-kinase (PI3K)/phospho-Akt (p-Akt) activity, affecting downstream signaling, reducing epithelial cell (EC) proliferation, and contributing to intestinal mucosal atrophy. We hypothesized that promoting Akt activity would prevent these changes. We used a novel Akt-activating peptide, TCL1 (a head-to-tail dimer of the Akt-binding domain of T-cell lymphoma-1), or an inactive mutant sequence TCL1G conjugated to a transactivator of transcription peptide sequence to promote intracellular uptake in total parenteral nutrition-fed animals. Sustaining phosphatidylinositol 3-kinase/phospho-Akt signaling in this fashion prevented the decline in proliferation and increase in epithelial cell apoptosis and a decline in the mammalian target of rapamycin pathway. This study defines Akt as central to total parenteral nutrition-induced mucosal atrophy and demonstrates efficacy of a novel cell-permeant Akt activator.

Total parenteral nutrition (TPN), or the removal of all enteral nutrition, is critical for patients who cannot tolerate enteral feeding. Despite its clinical importance, TPN administration is associated with a decline in epithelial cell (EC) proliferation, increase in EC apoptosis, and mucosal atrophy, and these physiological findings may well contribute to an increase in septicemia and infectious complications (14, 16, 17). In normal gastrointestinal mucosa, intestinal epithelial proliferation is crucial for maintaining normal crypt-villus architecture (5, 29).

The mechanisms that contribute to the loss of normal crypt-villus structures with TPN administration are incompletely understood and are most certainly multifactorial. Our laboratory has recently shown that TPN administration led to a downregulation of phosphatidylinositol 3-kinase (PI3K)/phospho-Akt (p-Akt) activity (6). This observation may well be a strong contributing factor to the loss of EC proliferation and increases in EC apoptosis observed with TPN administration (33). The PI3K/p-Akt signaling pathway is a major signaling pathway that contributes to cell growth and survival (19, 20). PI3K/p-Akt signaling also contributes to the activation of mammalian target of rapamycin (mTOR), which integrates signals from nutrients and energy status into the regulation of a variety of processes, including cellular metabolism via activation of mTOR (4, 21). This latter function integrates signals from nutrients and energy status into the regulation of a variety of processes, including cellular metabolism (4, 21).

To test the importance of p-Akt signaling in the development of TPN-associated loss of EC proliferation, a recently described promoter of p-Akt production was used in this study (23). TCL1 (a head-to-tail dimer of the Akt-binding domain of T cell lymphoma-1), which can promote the phosphorylation of p-Akt at two critical sites, was linked to a transactivator of transcription (TAT) sequence, which permitted the intracellular entrance of TCL1. We hypothesized that the use of this
novel promoter would prevent many of the observed changes in the intestinal epithelium with the use of TPN.

MATERIALS AND METHODS

Animals. C57BL/6J male, specific pathogen-free mice (10–11 wk old; Jackson Laboratory, Bar Harbor, ME) were maintained under temperature-, humidity- and light-controlled conditions. Mice were initially fed ad libitum with standard mouse chow and water and allowed to acclimate for 1 wk before surgery. During the administration of intravenous solutions, mice were housed in metabolic cages to prevent coprophagia. The studies were approved by our University of Michigan Animal Use Committee (no. 7703).

Operative procedures and TPN. Cannulation and administration of TPN was identical to that previously described (15). After 24 h, mice were randomized to four groups: 1) controls consisting of enterally fed mice plus intravenous saline, 2) control + TAT1 consisting of enterally fed mice receiving TAT-TCL1 in intravenous saline (see below), 3) TPN + TCL1G (control peptide), and 4) TPN + TCL1 (active peptide) group. Nitrogen delivery was controlled between each group (isonitrogenous). Caloric delivery was also kept isocaloric between groups (15). All animals were killed at 7 days using CO2.

Peptide-mediated activation of Akt. A novel peptide was used to promote p-Akt activity (22, 23). This peptide links a polybasic TAT sequence, which allowed intracellular permeability to the Akt-binding domain of the endogenous Akt coactivator, TCL1 (14). This peptide was previously reported to stimulate two main Akt phosphorylation sites, which can result in maximal activity (308 in the kinase domain and Serine-473 in the regulatory domain). A control peptide TAT-TCL1G contained point mutation and had minimal activity. Acute in vivo use of this peptide led to increased Akt activity and phosphorylation of both proximal (glycogen synthase kinase, GSK-3β) and distal (p70S6k) as well as extracellular regulated kinase (ERK). The present study examined a chronic dosing regimen. Several doses (0.5 mg/day, 0.2 mg/day, and 0.1 mg/day) were examined for tolerance, as the use of TAT peptide may mediate a high cell membrane permeability, which could result in potential toxicity at high doses (22, 31, 34). No mortality (100% survival) was observed for the animals receiving 0.1 mg of peptide; therefore 0.1 mg of the respective peptide was added to each 4.8 ml TPN solution daily beginning on the second day after placement of the TPN cannulas. Additionally, 1 mg peptide was administered intraperitoneally 3 h before euthanasia. An intraperitoneal route was selected to avoid introduction of air or bacteria into the intravenous line.

Real-time PCR. Mucosal scrapings were placed in TRIzol and homogenized, and RNA was extracted and purified as previously described. Oligomers were designed using an optimization program Primer Premier (www.premierbiosoft.com), and sequences of specific primers are described in Table 1. RT-PCR was performed using a

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Table 1. Primers used in study

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Fig. 1. Phosphorylated-Akt (p-AKT). A: shown are representative immunoblot bands for p-Akt (Ser473 and Thr308, respectively) and total Akt expression for the following groups: control, control given active peptide (TCL1, a head-to-tail dimer of the Akt-binding domain of T-cell lymphoma-1), total parenteral nutrition (TPN) plus nonactive peptide (TCL1G), and TPN plus TCL1. B: results are expressed as the mean fold change in p-Akt at Ser473 mRNA relative to total Akt. C: results are expressed as the mean fold change in p-Akt at Thr308 mRNA relative to total Akt. Note that p-Akt significantly decreased with TPN administration as shown in the TPN + TAT-TCL1G group. Administration of TAT-TCL1 to control mice did not affect p-Akt abundance; however, TAT-TCL1 in TPN mice partially prevented the decline in p-Akt (Ser473) (B). However, TAT-TCL1 did not affect p-Akt abundance at the Thr308 phosphorylation site (C). Results of each group represent an n = 4–6. #P < 0.05; **P < 0.001.
Rotor-Gene 6000 (Corbett Life Science, Sydney, Australia), and /H9252-actin was used as an internal control for normalization. Fold changes of target genes were calculated using comparative quantification to /H9252-actin.

Western immunoblotting. Intestinal epithelial cells were prepared by using a modification of Grossmann’s technique (13) and with immunoblotting techniques as previously described (26). The following antibodies (Ab) used included: mouse anti-/H9252-catenin Ab (clone IgG1; BD Transduction Laboratories, BD Biosources, San Jose, CA), rabbit anti-phosphorylated Akt1/2/3 (Ser 473 and Thr308; Santa Cruz Biotechnology, Santa Cruz, CA), rabbit anti-phosphorylated GSK-3/H9251/H9252 (Ser21/9), mouse anti-cyclin D1, rabbit anti-phospho-p70 S6 kinase, rabbit anti-4E binding protein 1 (4EBP1), and mouse anti-proliferating cell nuclear antigen (PCNA) (Cell Signaling Technology, Beverly, MA). Secondary antibody was the horseradish peroxidase conjugate of either goat anti-mouse or goat anti-rabbit IgG (Cell Signaling).

Immunohistochemistry and immunofluorescent staining. Zinc-formalin-fixed (10%) and paraffin-embedded tissue sections (5 μm) were stained using standard immunohistochemistry or immunofluorescent staining procedures, as previously described (8). The following primary antibodies and dilutions were used for immunohistochemistry: anti-Akt1/2/3 (Ser 473; at a 1:100 dilution, Santa Cruz), and anti-GSK-3β (Cell Signaling Technology, 1:50); biotinylated goat-anti-rabbit secondary antibody was used at a dilution of 1:200. The signal was amplified using streptavidin-horseradish peroxidase conjugate (Santa Cruz) and visualized with 3,3-diaminobenzidine substrate followed by hematoxylin. The following primary antibodies and dilutions were used for immunofluorescent staining: rabbit anti-Eukaryotic initiation factor 4EBP1 (1:200, Cell Signaling Technology), mouse anti PCNA (1:4,000, Cell Signaling Technology), mouse anti-/H9252-catenin (clone IgG1; BD Transduction Laboratories), and goat anti-mouse-FITC IgG; goat anti-mouse-Cy3 IgG1 and goat anti-rabbit-FITC IgG were used as corresponding secondary antibody. Additional staining was performed for active caspase-3 as a marker of apoptosis (20), using anti-rabbit cleaved caspase-3 (Cell Signaling Technology). Immunofluorescent localization of p-p70 S6 was not done, as current available antibodies do not stain intestinal mucosal tissue adequately. Results are expressed as the percentage of active caspase-3-positive staining cell numbers in total EC numbers per villi; 10 villi were counted in each mouse.

EC proliferation was determined using 5-bromo-2-deoxyuridine (BrdU) (50 mg/kg; Roche Diagnostic, Indianapolis, IN) as previously described (26). An index of the crypt cell proliferation rate was calculated as the ratio of the number of crypt cells incorporating BrdU to the total number of crypt cells. The total number of proliferating cells per crypt was defined as the mean of proliferating cells in 10 crypts for each mouse.

Data analysis. Data are expressed as the means ± SD. Statistical analyses employed Student’s t-test for comparison of two means and a one-way ANOVA for comparison of multiple groups. For ANOVA

Fig. 2. Abundance of phospho-glycogen synthase-kinase (p-GSK)-3β. p-GSK-3β was decreased with TPN, and this was prevented with TCL1 administration. A: representative immunoblot bands of p-GSK-3β and β-actin are shown for each study group. Note that p-GSK-3β is shown as the lower of the two bands, and p-GSK-3α is the upper band. B: summarized results of Western immunoblotting as expressed as the ratio of p-GSK-3β expression to β-actin. Note the decline in p-GSK-3β in the TPN + TCL1G group and a significant prevention of this decline in the TPN + TCL1 group. Results of each group represent an n = 4–6. *P < 0.01.

Fig. 3. Intestinal epithelial cell β-catenin protein expression by Western immunoblots. A: representative gels for β-catenin. B: summarized results of β-catenin protein expression as normalized to β-actin. Intestinal epithelial cell β-catenin protein abundance was significantly decreased in the TPN + TCL1G group compared with controls. This decline was partially prevented in the TPN + TCL1 group. Results of each group represent an n = 5. **P < 0.001.
a Bonferroni’s post hoc analysis to assess statistical differences between groups was performed. The Chi-square test was used for categorical data (Prism software; GraphPad Software, San Diego, CA). Statistical significance was defined as \( P < 0.05 \).

**RESULTS**

**TAT-TCL1 prevented TPN administration associate loss of p-Akt abundance.** TPN administration resulted in a significant reduction in the amount of activated Akt (Ser473) compared with the sham group (Fig. 1); the level of decline was similar to that previously described (8). Chronic administration of TAT-TCL1 (0.1 mg/day for 6 days) in the TPN solution led to a partial prevention in the decline of p-Akt (Ser473) abundance, whereas TPN mice treated with the Akt binding-deficient TAT-TCL1G showed a marked loss of pSer473 Akt abundance (Fig. 1B) compared with saline-treated controls. Enterally fed control mice administered the TAT-TCL1 peptide (0.1 mg/day for 6 days) had similar p-Akt abundance as saline-treated enteral control mice. The abundance of pThr308 Akt in TPN + TAT-TCL1G showed a marked decline compared with both control groups. There was no change in the abundance of pThr308 Akt in the TPN + TCL1 (Fig. 1C).

**TAT-TCL1 promotion of p-Akt abundance led to downstream signaling in the Wnt/β-catenin pathway.** The increased abundance of p-Akt does not necessarily reflect that Akt was able to transduce signals appropriately. The consequences of sustained p-Akt (Ser473) abundance in the TPN + TCL1 mice group were examined by determining whether proximal targets of p-Akt were phosphorylated. A classic p-Akt signaling pathway is the Wnt/β-catenin pathway. We began by examining GSK-3β and β-catenin expression.

Phospho-GSK-3β abundance was unaffected in the control + TCL1 group (Fig. 2; 0.62 ± 0.06 vs. 0.55 ± 0.13; control + saline vs. control + TCL1, respectively). P-GSK-3β was significantly \( P < 0.01 \) decreased (~2.5-fold) in the TPN + TCL1G group (0.23 ± 0.09). However, the loss of p-GSK-β was almost completely prevented \( P < 0.01 \) in the group of TPN mice treated with TCL1 (0.58 ± 0.10; TPN + TCL1 group; Fig. 2B). Immunohistochemical staining showed that p-GSK-3β was primarily located in the jejunal crypts; a marked decline in p-GSK-3β was observed in the crypts of TPN + TCL1G mice, and a partial prevention of this loss was seen in the TPN + TCL1 group (data not shown).

A similar pattern of expression was seen for β-catenin (Fig. 3). A loss of β-catenin was observed in the TPN + TCL1G group, and a prevention of this loss of β-catenin was found in the TPN + TCL1 group. Immunofluorescent imaging demonstrated an almost complete loss of β-catenin in the TPN + TCL1G group, whereas the TPN + TCL1 cohort exhibited an intermediate level of fluorescence (Fig. 6, see below).

**TAT-TCL1 promoted EC proliferation through activation of the T cell factor/lymphoid enhancer factor transcription factor cyclin D1.** The distal end of the β-catenin pathway is T cell factor (TCF)-4 mediated transcriptional activation. As a marked loss of β-catenin (nuclear and cytoplasmic) was detected in TPN + TCL1G mice, it was next determined whether this resulted in a loss of TCF-4 transcriptional activation by measuring the RNA abundance of two known TCF-4 target genes, c-myc and cyclin D1. Compared with both control groups after 7 days of TPN, there was a nearly threefold decline in mRNA and protein expression for cyclin D1 in the TPN + TCL1G group, and this decline was prevented in the TPN + TCL1 group (Fig. 4). c-Myc expression was also found to be decreased in the TPN + TCL1G group; however, no significant difference was observed with the use of TCL1 (data was not shown).

Cyclin D1 and c-myc are two major regulators of the progression of cells into the proliferative stage of the cell cycle (13, 31, 34). Therefore, the tissue-level functional consequences of TCL1-mediated sustained p-Akt abundance and activity were assessed. BrdU incorporation was assessed as a marker of proliferation.

Fig. 4. Cyclin D1 expression in intestinal epithelial cells. A: mRNA expression of cyclin D1. B: representative immunoblot gels for cyclin D1. C: summarized results of cyclin D1 protein expression. Cyclin D1 decreased significantly in the TPN + TCL1G group compared with controls. This decline was partially prevented in the TPN + TCL1 group. Results of each group represent an \( n = 5 \). \( * * P < 0.001 \), * \( P < 0.01 \).
TPN + TCL1G mice showed a marked decline in EC proliferation compared with both control groups (0.23 ± 0.06 vs. 0.49 ± 0.06 and 0.53 ± 0.12, TPN + TCL1G vs. control + saline and control + TCL1, respectively). TPN + TCL1 mice had a preservation of proliferation to nearly the same level as the control group (0.41 ± 0.07 TPN + TCL1), and the level was not significantly different than controls (Fig. 5F).

The BrdU incorporation data were supported by measuring PCNA expression by Western blot and immunohistochemistry. TAT-TCL1 did not affect PCNA expression in the control group (0.51 ± 0.07 vs. 0.53 ± 0.07 control + saline vs. control + TCL1, respectively), whereas PCNA expression was approximately fivefold lower in the TPN + TCL1G group (0.1 ± 0.05). A prevention in this loss of PCNA was noted in the TPN + TCL1 group (0.39 ± 0.08; Fig. 5, D and E). Immunofluorescent staining showed the number of PCNA-positive staining crypt cells (Fig. 5). Immunofluorescent intensity was markedly lost in the TPN + TCL1G group (Fig. 5B) compared with the control + TCL1 group (Fig. 5A), and this loss was partially prevented in the TPN + TCL1G group (Fig. 5C). No difference was noted between control + saline vs. control + TCL1 groups (data not show).

**TAT-TCL1 administration prevented TPN-associated changes in the mTOR pathway.** The scope of the activity of p-Akt was assessed by determining the effects on the mTOR pathway, p70 S6 kinase (pp70S6K; or PP70), and expression/localization of 4EBP1, proteins that mediate mTOR signaling and mRNA translation (26). Administration of TAT-TCL1 did not affect the phosphorylation of p70S6K in the control group (0.22 ± 0.05 vs. 0.25 ± 0.05; control + saline vs. control + TCL1, respectively). A significant (P < 0.05) loss of pp70S6K expression was detected in TPN + TCL1G group (0.10 ± 0.03), whereas a retention of normal pp70S6K expression was measured in TPN mice treated with TCL1 (0.25 ± 0.09; P < 0.05 compared with the TPN + TCL1G group, Fig. 6).

Loss of PI3K/p-Akt signaling in intestinal EC was found to increase 4EBP1 expression and was associated with the dissociation of the β-catenin/4EBP1 complex. Immunofluorescent detection of 4EBP1 in histological sections revealed that most of the 4EBP1-positive cells were located at the bottom of the crypts in the TPN + TCL1G group, and this intensity was markedly increased compared with the control groups (control + TCL1, Fig. 7A). As a control, administration of TAT-TCL1G did not affect the 4EBP1 intensity in the control group (data not shown). Additionally, histological sections from control mice showed colocalization of 4EBP1 and β-catenin, which was lost in the TPN + TCL1G cohort. 4EBP1 signal intensity was decreased in the TPN + TCL1 group compared with TPN + TCL1G mice although still slightly higher than the control group. Treatment of TPN mice with TCL1 also preserved the membrane localization of β-catenin.

**TAT-TCL1 administration partially prevented TPN-associated epithelial cell apoptosis.** Another major physiological role of the PI3K/Akt signaling pathway is to inhibit apoptosis. Gut epithelial cell apoptosis is a relatively early event following the transition from enteral to parenteral nutrition; therefore epithelial cell apoptosis was determined in gut epithelial cells after 3
days. First, mRNA expression was measured for three members of the Bcl-2 family, including Bad, Bax, and Bcl-2. Both Bad and Bax expression increased significantly in the TPN + TFT1G group compared with controls (Fig. 7, A and B). There was no significant difference for Bcl-2 expression across the four groups (Fig. 7C).

Immunofluorescent staining for active caspase-3 was then performed on histological sections of small bowel. The result showed very low levels of active caspase-3 staining cells in the control + saline group (Fig. 7, D and G). Although not shown, this low caspase-3 activity was also observed in control + TCL1 mice (0.013 ± 0.005; caspase-3 EC per villi). The percentage of active caspase-3-positive staining cells was 10-fold higher (0.12 ± 0.04) in the TPN + TAT1G group, with most of the positive staining EC cells being located at the base of the villi (Fig. 7E). The percentage of active caspase-3-positive staining cells significantly decreased to 0.06 ± 0.02 in TPN + TCL1 group, a twofold decline compared with the TPN + TAT1G cohort (Fig. 7, F and G).

**DISCUSSION**

Our laboratory has previously showed that the administration of TPN resulted in a loss of intestinal EC p-Akt phosphorylation (19). In this former study, glutamine was used to prevent the loss of p-Akt expression in our TPN model, and glutamine partially prevented the observed loss of EC proliferation with TPN. Glutamine is known to stimulate epithelial proliferation (30, 35). However, although glutamine has been shown to modulate the PI3K/Akt pathway (18), several reports have shown that glutamine can also affect many other intracellular signaling pathways in addition to PI3K/Akt (3, 6, 7). To determine the relevance of this TPN-associated loss in p-Akt, the present study used a novel promoter of phosphorylation, TAT-TCL1 (23). This peptide prevented a significant portion of the TPN-induced loss of Akt phosphorylation, which resulted in 1) the partial prevention in TPN-induced loss of EC proliferation, 2) the attenuation of TPN-induced increase in EC apoptosis, and 3) the maintenance of mTOR activation. Taken together these data suggest that the PI3K/p-Akt signaling pathway is critical for maintaining gut integrity, and many of the GI histopathological findings with TPN administration can be explained by loss of PI3K/p-Akt activity.

Akt is known to be a key regulatory pathway in several disease states, including inflammatory bowel disease and in other acute injuries such as myocardial and cerebral ischemia/reperfusion. Thus there has been an intense interest in understanding how to modulate this critical signaling pathway (9). The use of TCL1 in our work is one such approach. The βA strand of TCL1 forms a homodimer, each face of which then
immunofluorescent staining. Note the marked increase in the number and density of caspase-3-positive staining cells in the TPN group (B) compared with the control group (A). Summation of caspase-3 activity is expressed as the percentage of active caspase-3-positive epithelial cells (F). Summation of caspase-3 activity is expressed as the percentage of active caspase-3-positive epithelial cells (G).

binds one molecule of Akt (1, 23). TCL1 may then aid in recruiting Akt to the membrane where the activating kinases PDK1 and PDK2 are located. Alternative mechanisms include bringing two molecules of Akt into proximity to facilitate transactivation, or that the complex provides steric shielding for the phosphorylation sites, rendering them less accessible to the phosphatases that inactivate Akt (for example, protein phosphatase 2A). This approach, and linking TCL1 to the transfer domain of the HIV-1 protein TAT (27), has shown that Akt can be activated in vivo in a specific, receptor-independent fashion in a nontransgenic acute septic mouse model (23). However, this previous work used this TAT-TCL1 peptide for a relatively short period of time. In our present study, TAT-TCL1 and its nonactive peptide were used in vivo in a much more chronic process (1-wk period). As high doses of TAT may be toxic, we carefully titrated the dosing to 0.1 mg/day, which led to excellent mouse survival during TPN administration. Clearly, future adjustment of this dosing may be needed to better understand this complex signaling pathway in our TPN model.

In our present study, both p-Akt at Ser473 and at Thr308 were downregulated with TPN administration using the control peptide, TAT-TCL1G, whereas, in the TPN + TCL1 group, there was significantly increased abundance of p-Akt, as detected by Western immunoblotting and immunohistochemistry staining at Ser473. It was interesting to note that there was no significant change in the abundance at the Thr308 phosphorylation site. This differential expression may not be entirely surprising. In McDunn’s initial work (23), it also appeared that use of TCL1 on serum-starved Jurkat cells led to a far greater abundance of Ser473 phosphorylation compared with the Thr308 site. Furthermore, due to the chronic nature of the present study, it is also possible that we may have missed an earlier peak in the abundance of p-Akt at the Thr308 site. Our present work emphasizes that relatively small levels of sustained p-Akt abundance have tremendous potential to sustain cell viability and function. The mechanisms by which TCL1 differentially affected each study group is not fully known. It was interesting that TCL1 exerted minimal action in control mice and marked effect in the TPN group. It is possible that the action of TCL1 is to prevent p-Akt activity from declining below a certain level. It is also possible that TCL1 exerts a differential effect on normal proliferating epithelial cells (minimal action) compared with those epithelial cells with low levels of p-Akt activity (proliferative effect).

TPN-associated loss of p-Akt has been linked to a down-regulation in p-GSK-3β and β-catenin (8). In our present study a decline in p-GSK-3β and β-catenin was observed in the TPN + TCL1G group, and this was prevented in the TPN + TCL1 group. Additionally, the use of this active compound led to a prevention in the decline in EC proliferation observed in TPN mice receiving TAT-TCL1G. Additionally, this strongly supports the fact that TPN-associated intestinal mucosal atrophy is due to a loss of PI3K/Akt activity. This appears to be supported by a previous study that showed that loss of p-Akt has been observed in mice given an elemental diet (e.g., glucose, electrolytes, and water), with p-Akt expression rapidly rebounding with administration of a solid diet (28). Additionally, such actions could clearly influence downstream factors including the fate of intracellular β-catenin and cell proliferation.

In addition, Akt has been reported to be involved in the phosphorylation and activation of mTOR at Ser2448, and then affecting downstream signals PP70s6K and 4EBP1. Therefore, to further determine the functional affects of sustaining p-Akt abun-
dance in our TPN model, this pathway was also studied. TPN administration in mice given TAT-TCL1G led to a decline in PP70S6K expression and activated 4EBP1 expression. The administration of TAT-TCL1 to TPN mice prevented both of these changes. The mTOR pathway is tightly related to cell growth and the G1 cell cycle and contributes to cell proliferation (25, 27a). With TPN, no colocalization was observed between β-catenin and 4EBP1 in immunofluorescent images. This suggested that both Wnt/β-catenin and mTOR pathways are adversely affected in the TPN mice model, and that inhibition of both pathways contributes to poor EC proliferation in our TPN model.

Although the mTOR pathway has not been previously explored in TPN model, because of the known association of mTOR with the modulation of metabolism, such changes are intriguing. The rictor-mTOR complex phosphorylates Akt/PKB on Ser473 in the hydrophobic motif, which may facilitate the phosphorylation by PDK1 of the activation loop of Akt/PKB on Thr308. How the rictor-mTOR complex is regulated is unknown. In its nonphosphorylated state 4EBP1 represses cap-dependent mRNA translation by binding to the elF-4E cap binding protein and preventing it from interacting with the elF-4G protein. Phosphorylation of 4EBP1 by raptor-mTOR releases elF-4E to restore cap-dependent translation, which is particularly important for the translation of mRNA (10). It has been reported that inhibition of mTOR by rapamycin markedly impairs 4EBP1 phosphorylation and blocks its release from elF4E(2). Furthermore, phosphorylation of 4EBP1 is strongly modulated by the PI3K/Akt pathway as well as by nutrients/amino acids, both upstream regulators of mTOR signaling (11, 24, 32). In our study, both of mTOR downstream factors were inhibited with TPN administration, and stimulation of p-AKT activity prevented these changes.

It has been reported that PI3K elicits its antiapoptotic activity through the activation of the serine/threonine kinase Akt. Presently, the precise downstream mechanisms by which the activation of p-Akt leads to a decline in epithelial cell apoptosis is unknown. Activated Akt can phosphorylate the proapoptotic protein Bad, allowing Bad to associate with 14–3–3 and dissociation from Bcl-XL, thereby freeing it to exert its antiapoptotic function (35). PI3K/Akt signals can also exert an antiapoptotic action on gastric epithelial cells in vitro and in vivo through Bax, Bcl-2, and inhibition of caspase-3 activity(36). In our present study, the inhibition of caspase-3 activity(36). In our present study, the Wnt/β-catenin and mTOR pathways are adversely affected in the TPN mice model, and that inhibition of both pathways contributes to poor EC proliferation in our TPN model.

In conclusion, TPN led to a marked decline in p-Akt abundance. By sustaining p-Akt with TAT-TCL1, a partial prevention in the loss of downstream signaling, including preservation of EC proliferation, reduction in apoptosis, and preserved mTOR signaling was observed in a TPN model. These results suggest that changes in the p-Akt signaling pathway may well be a major mechanism that contributes to many of the intestinal EC derangements associated with TPN administration. New strategies that can sustain phosphorylation of Akt may have clinical relevance to patients receiving parenteral nutrition.

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DISCLOSURES
No conflicts of interest are declared by the authors.

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