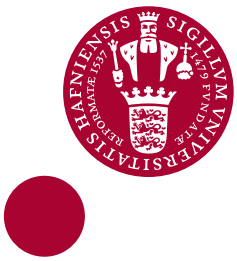

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Glucagon-Like Peptide-1 Is a Marker of Systemic Inflammation in Patients Treated with High-Dose Chemotherapy and Autologous Stem Cell Transplantation

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A B S T R A C T

Autologous stem cell transplantation (ASCT) is challenged by side effects that may be propagated by chemotherapy-induced mucositis, resulting in bacterial translocation and systemic inflammation. Because gastrointestinal damage appears as an early event in this cascade of reactions, we hypothesized that markers reflecting damage to the intestinal barrier could serve as early predictive markers of toxicity. Glucagon-like peptide-1 (GLP-1), a well-known regulator of blood glucose, has been found to promote intestinal growth and repair in animal studies. We investigated fasting GLP-1 plasma levels in 66 adults undergoing ASCT for lymphoma and multiple myeloma. GLP-1 increased significantly after chemotherapy, reaching peak levels at day +7 post-transplant (median, 8 pmol/L [interquartile range, 4 to 12] before conditioning versus 10 pmol/L [interquartile range, 6 to 17] at day +7; $P = .007$). The magnitude of the GLP-1 increase was related to the intensity of conditioning. GLP-1 at the day of transplantation (day 0) was positively associated with peak C-reactive protein (CRP) levels (46 mg/L per GLP-1 doubling, $P < .001$) and increase in days with fever (32% per GLP-1 doubling, $P = .0058$). Patients with GLP-1 above the median at day 0 had higher CRP levels from days +3 to +10 post-transplant than patients with lower GLP-1 ($P \leq .041$) with peak values of 238 versus 129 mg/L, respectively. This study, which represents the first clinical investigation of fasting GLP-1 in relation to high-dose chemotherapy, provides evidence that GLP-1 plays a role in regulation of mucosal defenses. Fasting GLP-1 levels may serve as an early predictor of systemic inflammation and fever in patients receiving high-dose chemotherapy.

INTRODUCTION

High-dose chemotherapy with autologous stem cell transplantation (ASCT) is a potentially curative treatment of many hematologic malignancies [1] but is challenged by severe side effects and complications such as mucositis, organ toxicity, and infections [2,3]. In most patients undergoing ASCT, varying degrees of systemic inflammation are seen during the first couple of weeks after conditioning with chemotherapy. This inflammatory response is initiated because of chemotherapy-induced injury to the mucosal barrier of the digestive tract [4-8], which increases intestinal permeability and translocation of bacterial products into the bloodstream [5,7,9,10]. Many previous studies have suggested that this inflammatory response plays a key role in induction of treatment-related complications and results in poor prognosis [11-16].

C-reactive protein (CRP) is a reliable marker for systemic inflammation after chemotherapy [17] and has been found to reach maximum levels about 7 to 14 days after infusion of stem cells [9,12,18,19]. This peak coincides with maximum intensity of mucositis as measured by clinical scoring, increased intestinal permeability, and a decline in citrulline, a marker of functioning enterocytes [4,12,20]. Importantly, the magnitude of the CRP response is associated with severe complications such as mucositis, pneumonia, bacteremia, *Clostridium difficile* colitis, and other infections, with potentially fatal outcome in patients undergoing ASCT [12,21]. However, the

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degree of inflammation is quite variable between patients, even among patients receiving the same conditioning regimen. It is not well understood why some patients are more prone to respond with severe inflammation than others, and we have at present no biomarkers that with sufficient precision can predict the inflammatory response in individual patients, limiting the possibility of personalized prophylaxis with antibiotics and other early interventions.

Continued proliferation and regeneration of intestinal epithelium is essential for maintenance of the mucosal barrier and involves peptide hormones produced in the gut [22,23]. Glucagon-like peptide- 1 (GLP-1) and GLP-2 are hormones co-secreted from intestinal enteroendocrine L cells in response to food intake and play a major role in regulating blood glucose through stimulation of insulin secretion (GLP-1) and promotion of nutrient absorption via expansion of the mucosal epithelium (GLP-2) [24-26]. However, increased secretion of both hormones is also seen after intestinal injury induced by chemotherapy in mice and rats [27-30] as well as in gut ischemia and inflammatory bowel disease in humans [31,32]. Importantly, GLP-2 and/or GLP-1 were found to have a trophic effect on the small intestine in several studies in rodents [33-37]. Additionally, GLP-2 has been shown to stabilize the integrity of intestinal epithelial barrier [38-41], and both GLP-1 and GLP-2 administration reduces chemotherapy-induced intestinal injury [27,42-45].

Based on these studies we hypothesized that elevated levels of GLP-1 could be an early marker of gut injury and potentially predict treatment-related complications in patients undergoing high-dose chemotherapy. The present study represents the first investigation of fasting GLP-1 plasma levels at consecutive time points in patients treated with high-dose chemotherapy followed by ASCT. Our findings indicate that increased levels of GLP-1 may serve as an early predictor of systemic inflammation and infection in these patients.

METHODS

Study Population

We prospectively included 66 patients (ages 20 to 72 years) undergoing ASCT at University Hospital Rigshospitalet, Copenhagen, Denmark from February 2015 to October 2016. Written and oral informed consent was obtained for all included patients. The study was approved by the local ethics committee (H-7-2014-016) and conducted in accordance with the Declaration of Helsinki. Inclusion criteria were patients scheduled for ASCT, age older than 18 years, and patient informed consent. Of 68 eligible patients, 2 were lost to follow-up before 3 weeks after transplantation due to transfer to other hospitals and therefore were excluded from the study.

The choice of pretransplant conditioning was based on the diagnosis in accordance with international guidelines, with all patients with myelomas receiving conditioning regimen 1 and all patients except 2 with lymphoma receiving conditioning regimen 2 (Table 1). All patients received oral antimicrobial prophylaxis consisting of ciprofloxacin, coamoxiclav, and fluconazole from day +4. Intravenous treatment with piperacillin/tazobactam or meropenem antibiotics was given based on daily measurements of body temperature and was administered according to a standardized regimen during days with fever (>38.5°C) until body temperature declined below 38.5°C. Body temperature was measured daily.

Inflammatory Parameters

CRP was monitored routinely in all patients every other day as a minimum during the first 3 weeks post-transplantation, and extra measurements were taken at the discretion of the physician in charge. When more than 1 measurement per day per patient was available, we calculated the mean to represent the CRP level of that day. Post-transplantation CRP_{max} was defined as the maximum CRP value from day +1 to day +21. CRP was analyzed using Modular P Module (Roche, Basel, Switzerland) (upper normal limit, 10 mg/L) at the Department of Clinical Biochemistry, University Hospital Rigshospitalet. Blood cultures were routinely collected on all patients with fever before initiation of i.v. antibiotic treatment, and results were registered from day −14 to day +30.

Table 1
Patient Characteristics and Treatment Modalities (N = 66)

Characteristics	Value
Median age, yr (range)	56 (20-72)
Sex	
Male	41 (62)
Female	25 (38)
Diagnosis	
Multiple myeloma	33 (50)
Non-Hodgkin lymphoma	29 (44)
Hodgkin lymphoma	3 (5)
Plasma cell leukemia	1 (2)
Conditioning regimen	
Melphalan high dose +/- carfilzomib/bortezomib	33 (50)
BEAM	30 (45)
Ara-C + CP + VP16 + thiotepa + bortezomib + lenalidomide	1 (2)
BCNU + thiotepa + rituximab	2 (3)

Values are n (%) unless otherwise defined. Ara-C indicates cytarabine; BCNU, carmustine; VP16, etoposide; CP, cyclophosphamide; BEAM, Ara-C + BCNU + VP16 + melphalan.

Quantification of GLP-1

Blood samples were collected at 5 time points during ASCT: before initiation of the conditioning regimen (baseline), at the day of transplantation before stem cell infusion (day 0), and at days +7, +14, and +21 post-transplantation. EDTA anticoagulated blood was centrifuged within 2 hours after collection, and plasma was isolated and stored at −80°C until analysis.

The plasma concentration of GLP-1 was measured in duplicates using a total GLP-1 ELISA (cat. no. 10-1278-01; Mercodia, Uppsala, Sweden) according to the manufacturer's instructions. This ELISA measures both active GLP-1 (7-36) amide and the degraded isoform GLP-1 (9-36) amide and reflects the secretion of GLP-1 because amidated isoforms of GLP-1 are highly predominant in humans [46]. Measurement range was .9 to 940 pmol/L.

Statistical Analyses

The associations of GLP-1 over time and with patient-specific characteristics (conditioning regimen, age, sex) were analyzed with a mixed model with a compound symmetry covariance matrix, and the significance was assessed with a Wald test with the degrees of freedom calculated using the Satterthwaite approximation. GLP-1 was log-transformed due to its skewness. Similarly, when investigating the association between CRP over time and dichotomized GLP-1 level at day 0. The Mann-Whitney U−test was used for comparison of continuous variables between groups. Simple and adjusted linear regression models were used to determine the association between GLP-1 day 0 (log 2-transformed) and CRP and negative binomial regression models to determine the association with days with fever. In subanalyses where conditioning was included as a covariate, only the 63 patients in either group 1 (high-dose melphalan, with or without bortezomib or carfilzomib) or group 2 (cytarabine in combination with carmustine, etoposide, and melphalan) were included.

A 2-sided $P < .05$ was considered statistically significant. All statistical analyses were performed using R statistical software version 2.15.3 (R Foundation for Statistical Computing, Vienna, Austria) and R*Studio* (RStudio, Boston, MA).

RESULTS

Clinical characteristics of the included patients are listed in Table 1.

GLP-1 Levels

The median GLP-1 level before conditioning for all included patients was 7.7 pmol/L (interquartile range, 4.3 to 11.5), which is within the range of the 95% confidence interval (CI) of fasting levels of 774 healthy individuals (1 to 21 pmol/L) [47]. Overall, the association between GLP-1 levels and measurement time points was significant ($P = .010$), and GLP-1 levels increased significantly from before conditioning to day 0 (33%; 95% CI,3 to 70; $P = .027$) and peaked at day +7 (41%; 95% CI, 10 to 81; $P = .0073$) (Figure 1A).

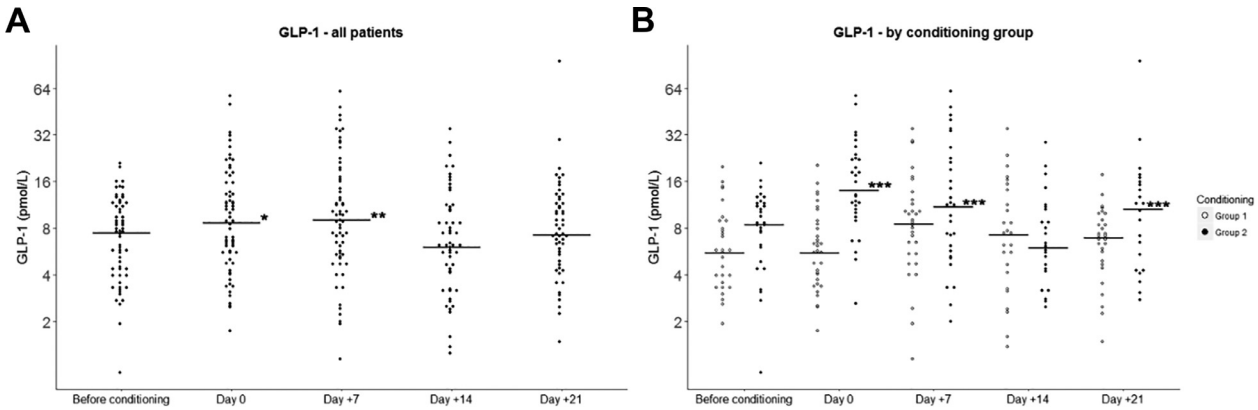

Figure 1. GLP-1 levels during ASCT from before start of conditioning until 21 days post-transplant. Day 0 refers to the day of transplantation after conditioning with high-dose chemotherapy. Horizontal line indicates median GLP-1. (A) All included patients. Statistical evaluation indicates GLP-1 levels compared with preconditioning levels (*P = .03; **P = .007). (B) Patients stratified by conditioning group. Patients conditioned with cytarabine in combination with carmustine, etoposide, and melphalan (group 2) had generally higher GLP-1 levels than patients treated with high-dose melphalan with or without bortezomib or carfilzomib (group 1). Statistical evaluation indicates differences between the 2 conditioning groups (***P < .001 [day 0]; P = .048 [day +7]; P = .03 [day +21]).

GLP-1 level before conditioning was not associated with sex (P = .38), age (P = .11), or diagnosis (P = .061). GLP-1 was significantly associated with conditioning group (P < .0001) with levels at days 0, +7, and +21 significantly higher in conditioning group 2 (patients with lymphoma) than in group 1 (patients with multiple myeloma) (155% [95% CI, 78 to 465; P < .0001], 44% [95% CI, .3 to 108; P = .048], and 54% [95% CI, 2 to 131; P = .03], respectively) (Figure 1B). Moreover, overall GLP-1 was significantly lower for older patients (11% per 10 years; 95% CI, 3 to 18; P = .013) but was not associated with sex (P = .35).

Inflammatory Response

Of the 48 patients with CRP measured at day 0, 44 (92%) had CRP day 0 within normal range (≤10 mg/L). Post-transplantation median CRP increased and reached a peak at day +9 after transplantation (Figure 2A), with daily median levels from day +2 and onward significantly higher than CRP day 0 (all P < .0017). Median post-transplant CRP_{max} was 181 mg/L (interquartile range, 61 to 266).

Fifty-two patients (79%) experienced fever > 38.5°C during the first 3 weeks post-transplant. The median duration of fever was 8 days (interquartile range, 3 to 12). At day +11 the highest number of patients experiencing fever was seen (47 [71%]) (Figure 2B).

GLP-1 and Inflammation

We investigated associations between GLP-1 and CRP and fever. A doubling in GLP-1 at day 0 was associated with an increase of 46 mg/L in CRP_{max} (95% CI, 24 to 68; P = .00018) (Figure 3A) and an increase of 32% in days with fever (95% CI, 8 to 62; P = .0058) (Figure 3B). When adjusting for conditioning group the associations with GLP-1 at day 0 remained significant (P = .024 and P = .042, respectively). In simple regression analyses, conditioning group 2 had on average 80 mg/L higher CRP_{max} compared with group 1 (95% CI, 22 to 138; P = .0091) and almost twice as many days with fever (96%; 95% CI, 27 to 201; P = .022). By comparing CRP in 2 groups stratified by the median values of GLP-1 at day 0 (9 pmol/L), we could demonstrate higher levels of CRP from days +3 to +10 in those with high GLP-1 values on day 0 (all $P \leq .041$) (Figure 4).

Infections

Only 2 patients presented with a positive blood culture (both post-transplant), which did not allow investigation of association between documented infections and GLP-1 levels.

Survival

Nine patients (14%) died during 1 year of follow-up, and 2 of these died because of treatment-related complications. These limited numbers did not allow conclusions regarding prediction of survival by GLP-1 measurements.

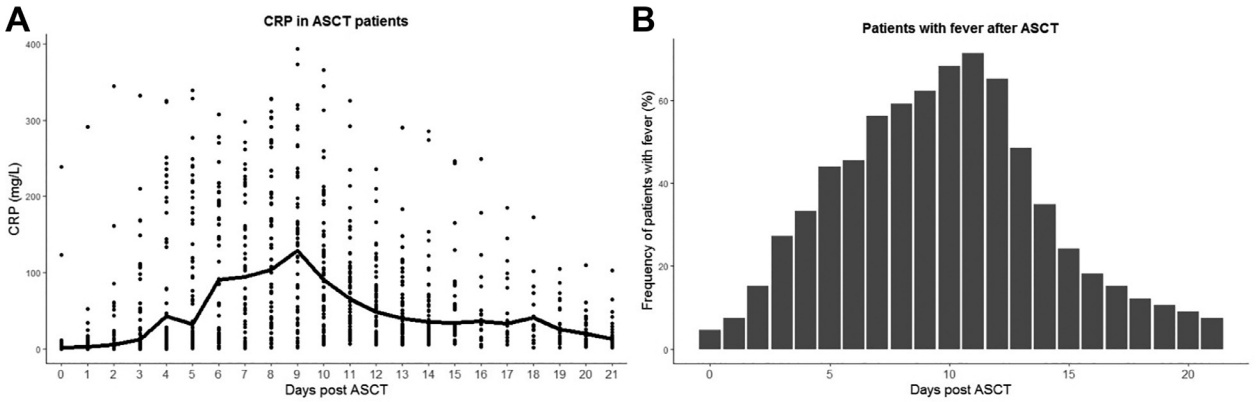

Figure 2. CRP and fever during treatment. CRP levels (A) and frequency of patients with fever (B) after high-dose chemotherapy and stem cell infusion. Black line in A indicates median CRP level.

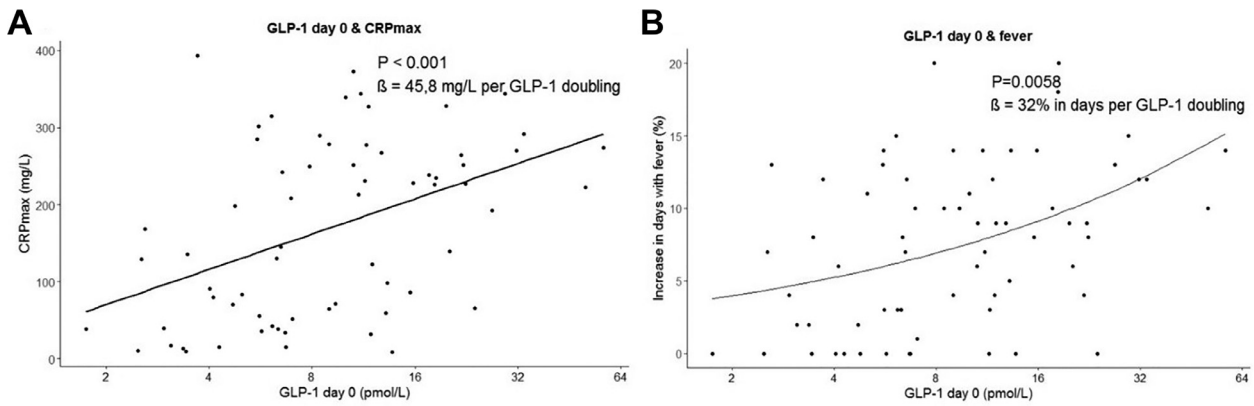

Figure 3. GLP-1 day 0 associations with inflammation after transplantation. Associations between GLP-1 levels at day 0 and inflammatory response: CRP_{max} (A) and days with fever (B) after ASCT. *P* values by simple linear regression and negative binomial regression analyses showed a significant association between GLP-1 levels at day 0 and inflammatory response.

DISCUSSION

In this study we investigated fasting GLP-1 levels in the early toxic phase in patients undergoing ASCT after high-dose chemotherapy. Our data indicate that fasting levels of GLP-1 are increased after chemotherapy, peaking around day +7 after the transplant, and, importantly, our data indicate that high levels of GLP-1 shortly after the completion of high-dose chemotherapy are predictive of subsequent increases in CRP during the first 3 weeks after transplantation.

GLP-2 is a well-known growth factor for intestinal epithelium, stimulating proliferation of crypt cells while inhibiting apoptosis of enterocytes in the small intestine [26,33,34,48-50]. A similar trophic effect of GLP-1 has been suggested by studies on rats [35,51] and mice [36,52] showing small intestinal and colonic growth after treatment with GLP-1 analogues. Furthermore, endogenous GLP-1 contributes to intestinal epithelial recovery after chemotherapy in mice [27], whereas loss of the GLP-1 receptor increases the severity of intestinal injury [53].

GLP-1 may also act as an anti-inflammatory peptide attenuating both local and systemic inflammation [54,55] by improving mucosal integrity through interactions with intestinal intraepithelial lymphocytes, found to express functional GLP-1 receptors [53,56]. In addition, GLP-1 has stimulatory effects on innate immune mechanisms, including production of alpha-defensin by Paneth cells [57]. GLP-1 treatment has shown anti-inflammatory effects by suppressing proinflammatory cytokines such as tumor necrosis factor-α, IL-6, and IL-1β in intestinal mucosa in mice with colitis [58-60] and in humans with diabetes type 1 and 2 and psoriasis [61-63]. Based on these findings, it may be hypothesized that increased level of GLP-1 in response to chemotherapy represents a protective

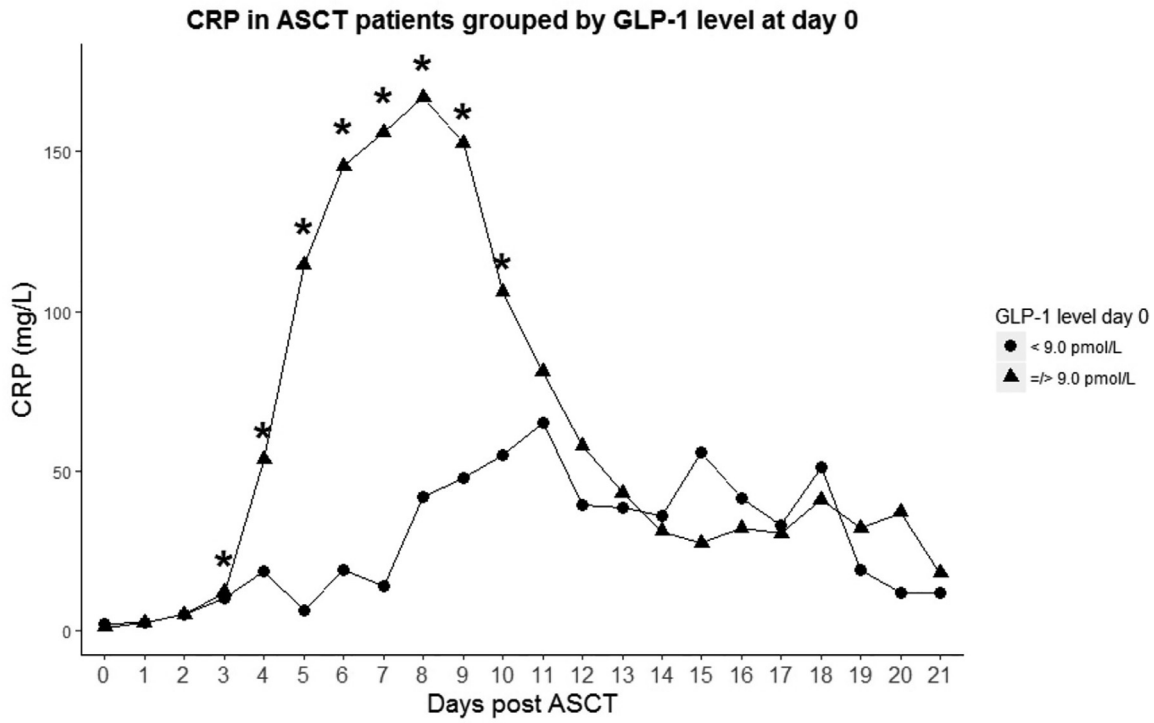

Figure 4. CRP after transplantation by GLP-1 increase at day 0. Median CRP levels after ASCT in 2 groups stratified according to the median levels of GLP-1 at day 0. Asterisk indicates statistical difference in CRP levels between the 2 groups (all $P \leq .041$).

reactive mechanism that may help to limit tissue damage and fasten recovery of epithelial integrity, thereby limiting inflammation.

To our knowledge we are the first to explore fasting GLP-1 levels in human subjects after chemotherapy treatment. However, our findings are in line with findings by Kissow et al. and others showing increased levels of both GLP-1 and GLP-2 after chemotherapy in rats [27] and mice [28-30]. Other studies found increased GLP-1 or GLP-2 levels after nonchemotherapy-related gut injury in humans [31,32] and animals [31], lending support to the notion that these hormones are secreted in response to intestinal injury.

Further evidence of such a protective effect is found in studies by Hytting et al. [30] showing that ablation of L cells in chemotherapy-treated mice leads to severe mucositis and insufficient intestinal healing, whereas chemotherapy-treated mice with normal function of L cells show compensatory hyperproliferation of intestinal epithelial cells in association with a marked increase in GLP-2 secretion. This is in line with data from Kissow et al. [27] showing similar findings when antagonizing the GLP-1 receptor selectively. Furthermore, Hytting et al. showed that co-treatment with both GLP-1 and GLP-2 analogues was more effective than a single GLP-1 analogue for rebuilding intestinal epithelium and maintaining body weight in mice.

The mechanism behind the increase in GLP-1 after gut injury is not fully understood, but data from Lebrun et al. [31] suggest that innate responses to bacterial lipopolysaccharide (LPS) may play a role in the induction of GLP-1 secretion. Accordingly, enteroendocrine L cells are stimulated by LPS through interaction with Toll-like receptor 4 after gut injury, leading to a rapidly increased secretion of GLP-1 [31]. Other studies in experimental animals confirmed increased GLP-1 secretion in response to LPS [64,65], apparently mediated through proinflammatory cytokines including IL-6 [64,66] and tumor necrosis factor-α [31,64,67]. Likewise, in humans Lebrun et al. [31] found GLP-1 secretion after LPS administration in volunteers. These authors also reported increased GLP-1 levels after induction of ischemia in the human intestine in vivo, suggesting a close relationship between gut inflammation and GLP-1 secretion in humans.

Breaks in the mucosa after chemotherapy have been found to serve as portals of entry for microorganisms, leading not only to bacteremia but also to penetration of pathogen-associated molecular patterns and endogenous danger-associated molecular patterns, produced as a result of cell death [68-70]. The resulting inflammatory response has been found to be predictive of organ toxicity and transplant-related mortality in allogeneic hematopoietic SCT [5,14-16,20,71,72], and several studies confirm a relationship between chemotherapy-induced mucosal barrier injury and systemic inflammation [4,8,9,19,73]. Our finding of a positive association between CRP and GLP-1 is in line with these results. Altogether, these studies suggest that increased levels of GLP-1 during chemotherapy may be induced by LPS in combination with an initial release of inflammatory cytokines.

Individuals with type 2 diabetes mellitus have a diminished meal-related GLP-1 secretion [74], and we could speculate this lower protection from GLP-1 after meals makes the intestinal epithelium more vulnerable after chemotherapy, resulting in higher risk of mucositis and severe systemic inflammation. Furthermore, it is not known if a diminished meal-related GLP-1 secretion makes the L cells less responsive to other secretory stimuli as well, potentially making the diabetes diagnosis a confounder for our results. Eight patients in our cohort were diagnosed with type 2 diabetes at the time of transplant, and these patients did not differ from the rest of the study population in GLP-1 levels or degree of systemic inflammation.

Nutrient ingestion is the primary physiologic stimulatory signal for GLP-1 and GLP-2 secretion [25,75]. Previous studies in rats [48] and mice [76] showed that fasting for 1 or a couple of days resulted in atrophy of the small intestine but could be reversed by refeeding and that this refeeding adaptation was prevented upon antagonizing the GLP-2 receptor. Other studies found that intestinal atrophy seen in rodents and pigs after total parenteral nutrition [48,77] could be ameliorated by co-infusion of GLP-2 [41,77,78]. Because patients undergoing high-dose chemotherapy treatment often have low or no enteral nutrition for days during the first few weeks after transplantation, the direct toxic effects of chemotherapy could be aggravated through insufficient GLP-1 and GLP-2 secretion, leading to incomplete healing and regeneration of the intestinal epithelium. Accordingly, further studies should focus on the role of enteral feeding in maintaining gut integrity through induction of GLP-1 and GLP-2. The variation in GLP-1 levels seen both pre- and post-transplant, even after stratification into conditioning regimens, demonstrates that additional factors may influence fasting plasma GLP-1 levels in humans, indicating a need for further studies to fully understand the potential of GLP-1 as a prognostic marker in clinical settings.

In conclusion, our data indicate that high-dose chemotherapy leads to increased fasting levels of GLP-1 and that these are predictive of increased systemic inflammation, adding to the mounting evidence that GLP-1 plays an important role in regulation of the mucosal defense. GLP-1 should be further investigated as a potential early predictive biomarker of treatment-related morbidity.

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REFERENCES

1. Philip T, Guglielmi C, Hagenbeek A, et al. Autologous bone marrow transplantation as compared with salvage chemotherapy in relapses of chemotherapy-sensitive non-Hodgkin's lymphoma. *N Engl J Med*. 1995;333:1540–1545.
2. Reich G, Mapara MY, Reichardt P, Dörken B, Maschmeyer G. Infectious complications after high-dose chemotherapy and autologous stem cell transplantation: Comparison between patients with lymphoma or multiple myeloma and patients with solid tumors. *Bone Marrow Transplant*. 2001;27:525–529.
3. Vera-Llonch M, Oster G, Ford CM, Lu J, Sonis S. Oral mucositis and outcomes of autologous hematopoietic stem-cell transplantation following high-dose melphalan conditioning for multiple myeloma. *J Support Oncol*. 2007;5:231–235.
4. van der Velden WJFM, Herbers AHE, Feuth T, Schaap NPM, Donnelly JP, Blijlevens NMA. Intestinal damage determines the inflammatory response and early complications in patients receiving conditioning for a stem cell transplantation. *PLoS One*. 2010;5:e15156.

5. Kornblit B, Muller K. Sensing danger: toll-like receptors and outcome in allogeneic hematopoietic stem cell transplantation. *Bone Marrow Transplant*. 2017;52:499–505.
6. Blijlevens NMA, van't Land B, Donnelly JP, M'Rabet L, de Pauw BE. Measuring mucosal damage induced by cytotoxic therapy. *Support Care Cancer*. 2004;12:227–233.
7. Blijlevens NMA, Donnelly JP, de Pauw BE. Prospective evaluation of gut mucosal barrier injury following various myeloablative regimens for haematopoietic stem cell transplant. *Bone Marrow Transplant*. 2005;35:707–711.
8. Cooke KR, Hill GR, Gerbitz A, et al. Hyporesponsiveness of donor cells to lipopolysaccharide stimulation reduces the severity of experimental idiopathic pneumonia syndrome: potential role for a gut-lung axis of inflammation. *J Immunol*. 2000;165:6612–6619.
9. Levy O, Teixeira-pinto A, White ML, Carroll SF, Hematopoietic B. Endotoxemia and elevation of lipopolysaccharide-binding protein after hematopoietic stem cell transplantation. *Pediatr Infect Dis J*. 2003;22:978–981.
10. Donnelly JP, Dompeling EC, Meis JF, et al. Bacteremia due to oral viridans streptococci in neutropenic patients with cancer : cytostatics are a more important risk factor than antibacterial prophylaxis. *Clin Infect Dis*. 1995;20:469–470.
11. Takatsuka H, Takemoto Y, Yamada S, et al. Complications after bone marrow transplantation are manifestations of systemic inflammatory response syndrome. *Bone Marrow Transplantation*. 2000;(May);26:419–426.
12. Fassas A-T, Miceli M, Grazzlutti M, Dong L, Barlogie B, Anaissie E. Serial measurement of serum C-reactive protein levels can identify patients at risk for severe complications following autologous stem cell transplantation. *Leuk Lymph*. 2005;46:1159–1161.
13. McNeer JL, Kletzel M, Rademaker A, et al. Early elevation of C-reactive protein correlates with severe infection and nonrelapse mortality in children undergoing allogeneic stem cell transplantation. *Biol Blood Marrow Transplant*. 2010;16:350–357.
14. Schots R, Riet IVan, Othman TBen, et al. Post-transplant complications: an early increase in serum levels of C-reactive protein is an independent risk factor for the occurrence of major complications and 100-day transplant-related mortality after allogeneic bone marrow transplantation. *Bone Marrow Transplant*. 2002;30:441–446.
15. Schots R, Kaufman L, Riet I Van, et al. Monitoring of C-reactive protein after allogeneic bone marrow transplantation identifies patients at risk of severe transplant-related complications and mortality. *Bone Marrow Transplantation*. 1998;(February);22:79–85.
16. Schots R, Kaufman L, Riet I Van, et al. Proinflammatory cytokines and their role in the development of major transplant-related complications in the early phase after allogeneic bone marrow transplantation. *Leukemia*. 2003;17:1150–1156.
17. Rintala E, Irjala K, Nikoskelainen J. Value of measurement of C-reactive protein in febrile patients with hematological malignancies. *Eur J Clin Microbiol Infect Dis*. 1992;11:973–978.
18. Manian FA, Diseases I, John S. A prospective study of daily measurement of C-reactive protein in serum of adults with neutropenia. *Clin Infect Dis*. 1995;21:114–121.
19. Blijlevens NMA, Donnelly JP, DePauw BE. Inflammatory response to mucosal barrier injury after myeloablative therapy in allogeneic stem cell transplant recipients. *Bone Marrow Transplant*. 2005;36:703–707.
20. Pontoppidan PL, Jordan K, Carlsen AL, et al. Associations between gastrointestinal toxicity, micro RNA and cytokine production in patients undergoing myeloablative allogeneic stem cell transplantation. *Int Immunopharmacol*. 2015;25:180–188.
21. Ortega M, Rovira M, Almela M, de la Bellacasa JP, Carreras E, Mensa J. Measurement of C-reactive protein in adults with febrile neutropenia after hematopoietic cell transplantation. *Bone Marrow Transplant*. 2004;33:741–744.
22. Sonis ST. Pathobiology of mucositis. *Nat Rev Cancer*. 2004;20:11–15.
23. Sonis ST. A biological approach to mucositis. *J Support Oncol*. 2004;2:21–26.
24. Elliott RM, Morgan LM, Tredger JA, Deacon S, Wright J, Marks V. Glucagon-like peptide-1(7-36)amide and glucose-dependent insulinotropic polypeptide secretion in response to nutrient ingestion in man: Acute post-prandial and 24-h secretion patterns. *J Endocrinol*. 1993;138:159–166.
25. Orskov C, Wettergren A, Holst JJ. Secretion of the incretin hormones glucagon-like peptide-1 and gastric inhibitory polypeptide correlates with insulin secretion in normal man throughout the day. *Scand J Gastroenterol*. 1996;31:665–670.
26. Drucker DJ. Biological actions and therapeutic potential of the glucagon-like peptides. *Gastroenterology*. 2002;122:531–544.
27. Kissow H, Hartmann B, Holst JJ, Poulsen SS. Glucagon-like peptide-1 as a treatment for chemotherapy-induced mucositis. *Gut*. 2013;62:1724–1733.
28. Hirotani Y, Yamamoto K, Ikeda K, Arakawa Y, Li J, Tanaka K. Correlation between plasma glucagon-like peptide 2 levels and proliferative makers in small intestinal injury in rats induced by methotrexate administration. *Biol Pharm Bull*. 2006;29:2327–2330.
29. Kissow H, Viby NE, Hartmann B, et al. Exogenous glucagon-like peptide-2 (GLP-2) prevents chemotherapy-induced mucositis in rat small intestine. *Cancer Chemother Pharmacol*. 2012;70:39–48.
30. Hytting-andreasen R, Balk-m E, Hartmann B, Kissow H. Are essential for regeneration after acute intestinal injury in mice. *PLoS One* 2018;1–14.
31. Lebrun LJ, Lenaerts K, Kiers D, Drucker DJ, Lagrost L, Grober J. Enteroendocrine L cells sense LPS after gut barrier injury to enhance GLP-1 secretion. *Cell Rep*. 2017;1160–1168.
32. Xiao Q, Boushey RP, Cino M, Drucker DJ, Brubaker PL. Circulating levels of glucagon-like peptide-2 in human subjects with inflammatory bowel disease. *Am J Physiol*. 2000;278:R1057–R1063.
33. Tsai C-H, Hill M, Asa S, Drucker DJ. Intestinal growth-promoting properties of glukagon-like peptide-2 in mice. *Am Physiol Soc*. 1997;22:1023–1032.
34. Tsai CH, Hill M, Drucker DJ. Biological determinants of intestinotrophic properties of GLP-2 in vivo. *Am J Physiol*. 1997;272(3 Pt 1):G662–G668.
35. Simonsen L, Pilgaard S, Orskov C, et al. Exendin-4, but not dipeptidyl peptidase IV inhibition, increases small intestinal mass in GK rats. *Am J Physiol Liver Physiol*. 2007;293:G288–G295.
36. Kissow H, Hartmann B, Holst JJ, et al. Glucagon-like peptide-1 (GLP-1) receptor agonism or DPP-4 inhibition does not accelerate neoplasia in carcinogen treated mice. *Regul Pept*. 2012;179:91–100.
37. Drucker DJ, Erlich P, Asa SL, Brubaker PL. Induction of intestinal epithelial proliferation by glucagon-like peptide 2. *Proc Natl Acad Sci USA*. 1996;93:7911–7916.
38. Benjamin MA, McKay DM, Yang PC, Cameron H, Perdue MH. Glucagon-like peptide-2 enhances intestinal epithelial barrier function of both transcellular and paracellular pathways in the mouse. *Gut*. 2000;47:112–119.
39. Dong CX, Zhao W, Solomon C, et al. The intestinal epithelial insulin-like growth factor-1 receptor links glucagon-like peptide-2 action to gut barrier function. *Endocrinology*. 2014;155:370–379.
40. Scott RB, Kirk D, MacNaughton WK, Meddings JB. GLP-2 augments the adaptive response to massive intestinal resection in rat. *Am J Physiol*. 1998;275(5 Pt 1):G911–G921.
41. Lei Q, Bi J, Chen H, et al. Glucagon-like peptide-2 improves intestinal immune function and diminishes bacterial translocation in a mouse model of parenteral nutrition. *Nutr Res*. 2017;49:56–66.
42. Kissow H, Viby N-E, Hartmann B, et al. Exogenous glucagon-like peptide-2 (GLP-2) prevents chemotherapy-induced mucositis in rat small intestine. *Cancer Chemother Pharmacol*. 2012;70:39–48.
43. Boushey RP, Yusta B, Drucker DJ. Glucagon-like peptide (GLP)-2 reduces chemotherapy-associated mortality and enhances cell survival in cells expressing a transfected GLP-2 receptor. *Cancer Research*. 2001;61:687–693.
44. Tavakkolizadeh A, Shen R, Abraham P, et al. Glucagon-like peptide 2: a new treatment for chemotherapy-induced enteritis. *Journal of Surgical Research*. 2000;91:77–82.
45. Rasmussen AR, Viby NE, Hare KJ, et al. The intestinotrophic peptide, GLP-2, counteracts the gastrointestinal atrophy in mice induced by the epidermal growth factor receptor inhibitor, Erlotinib, and cisplatin. *Dig Dis Sci*. 2010;55:2785–2796.
46. Orskov C, Rabenhoj L, Wettergren A, Kofod H, Holst JJ. Tissue and plasma-concentrations of amidated and glycine-extended glucagon-like peptide-I in humans. *Diabetes*. 1994;43:535–539.
47. Færch K, Toreskov S, Vistisen D, et al. GLP-1 response to oral glucose is reduced in prediabetes, screen-detected type 2 diabetes, and obesity and ifluenced by sex: the ADDITION-PRO study. *Diabetes*. 2015;64:2513–2525.
48. Nelson DW, Murali SG, Liu X, Koopmann MC, Holst JJ, Ney DM. Insulin-like growth factor I and glucagon-like peptide-2 responses to fasting followed by controlled or ad libitum refeeding in rats. *Am J Physiol Regul Integr Comp Physiol*. 2008;294:R1175–R1184.
49. Chen J, Dong J, Li X, et al. Glucagon-like peptide-2 protects impaired intestinal mucosal barriers in obstructive jaundice rats. *World J Gastroenterol*. 2015;21:484–490.
50. Lee S, Lee J, Li KK, et al. Disruption of the murine Glp2r impairs paneth cell function and increases susceptibility to small bowel enteritis. *Endocrinology*. 2013;153:1141–1151.
51. Nozu T, Miyagishi S, Kumei S, Nozu R, Takakusaki K, Okumura T. Glucagon-like peptide-1 analog, liraglutide, improves visceral sensation and gut permeability in rats. *J Gastroenterol Hepatol*. 2018;33:232–239.
52. Koehler JA, Baggio LL, Brubaker PL, et al. GLP-1R agonists promote normal and neoplastic intestinal growth through mechanisms requiring article GLP-1R agonists promote normal and neoplastic intestinal growth through mechanisms requiring Fgf7. *Cell Metab*. 2015;21:379–391.
53. Yusta B, Baggio LL, Koehler J, et al. GLP-1R agonists modulate enteric immune responses through the intestinal intraepithelial lymphocyte GLP-1R. *Diabetes*. 2015;64:2537–2549.
54. Drucker DJ. The cardiovascular biology of glucagon-like peptide-1. *Cell Metab*. 2016;24:15–30.
55. Lee YS, Jun HS. Anti-inflammatory effects of GLP-1-based therapies beyond glucose control. *Mediators Inflamm*. 2016;2016:26–32.
56. Kedees MH, Guz Y, Grigoryan M, Teitelman G. Functional activity of murine intestinal mucosal cells is regulated by the glucagon-like peptide-1 receptor. *Peptides*. 2013;48:36–44.

57. Ayabe T, Satchell DP, Wilson CL, Parks WC, Selsted ME, Ouellette AJ. Secretion of microbicidal alpha-defensins by intestinal Paneth cells in response to bacteria. *Nat Immunol*. 2000;1:113–118.
58. Anbazhagan A, Thaqi M, Priyamvade S, et al. GLP-1 nanomedicine alleviates gut inflammation. *Nanomedicine*. 2017;179:95–105.
59. Bang-Berthelsen CH, Holm TL, Pyke C, et al. GLP-1 induces barrier protective expression in Brunner's glands and regulates colonic inflammation. *Inflamm Bowel Dis*. 2016;22:2078–2097.
60. Shiraki A, Oyama J, Komoda H, et al. The glucagon-like peptide 1 analog liraglutide reduces TNF-α-induced oxidative stress and inflammation in endothelial cells. *Atherosclerosis*. 2012;221:375–382.
61. Ceriello A, Novials A, Ortega E, et al. Glucagon-like peptide 1 reduces endothelial dysfunction, inflammation, and oxidative stress induced by both hyperglycemia and hypoglycemia in type 1 diabetes. *Diabetes Care*. 2013;36:2346–2350.
62. Ceriello A, Novials A, Canivell S, et al. Simultaneous GLP-1 and insulin administration acutely enhances their vasodilatory, antiinflammatory, and antioxidant action in type 2 diabetes. *Diabetes Care*. 2014;37:1938–1943.
63. Hogan AE, Gaoatswe G, Lynch L, et al. Glucagon-like peptide 1 analogue therapy directly modulates innate immune-mediated inflammation in individuals with type 2 diabetes mellitus. *Diabetologia*. 2014;57:781–784.
64. Kahles F, Meyer C, Möllmann J, et al. GLP-1 secretion is increased by inflammatory stimuli in an IL-6-dependent manner, leading to hyperinsulinemia and blood glucose lowering. *Diabetes*. 2014;63:3221–3229.
65. Nguyen AT, Mandard S, Dray C, et al. Lipopolysaccharides-mediated increase in glucose-stimulated insulin secretion: Involvement of the GLP-1 pathway. *Diabetes*. 2014;63:471–482.
66. Ellingsgaard H, Hauselmann I, Schuler B, et al. Interleukin-6 enhances insulin secretion by increasing glucagon- like peptide-1 secretion from L cells and alpha cells. *Nat Med*. 2011;17:1481–1489.
67. Gagnon J, Sauvé M, Zhao W, et al. Chronic exposure to tumor necrosis factor α impairs secretion of glucagon-like peptide-1. *Endocrinology*. 2015;156:3950–3960.
68. Alikhani M, Alikhani Z, He H, Liu R, Popek BI, Graves DT. Lipopolysaccharides indirectly stimulate apoptosis and global induction of apoptotic genes in fibroblasts. *The Journal of Biological Chemistry*. 2003;278:52901–52908.
69. Yang H, Tracey K. Targeting HMGB1 in inflammation Huan. *Biochim Biophys Acta*. 2010;25:368–379.
70. Kawai T, Akira S. The role of pattern-recognition receptors in innate immunity: Update on toll-like receptors. *Nat Immunol*. 2010;11:373–384.
71. Jordan K, Pontoppidan P, Uhlving HH, et al. Gastrointestinal toxicity, systemic inflammation, and liver biochemistry in allogeneic hematopoietic stem cell transplantation. *Biol Blood Marrow Transplant*. 2017;23:1170–1176.
72. Haastrup E, Andersen J, Ostrowski SR, et al. Soluble urokinase plasminogen activator receptor during allogeneic stem cell transplantation. *Scand J Immunol*. 2011;73:325–329.
73. Elting LS, Cooksley C, Chambers M, Cantor SB, Manzullo E, Rubenstein EB. The burdens of cancer therapy: clinical and economic outcomes of chemotherapy-induced mucositis. *Cancer*. 2003;98:1531–1539.
74. Toft-Nielsen M-B, Damholt MB, Madsbad S, et al. Determinants of the impaired secretion of glucagon- like peptide-1 in type 2 diabetic patients. *The Journal of Clinical Endocrinology and Metabolism*. 2001;86:3717–3723.
75. Elliott RM, Morgan LM, Tredger JA, Deacon S, Wright J, Marks V. Glucagon-like peptide-1 (7-36)amide and glucose-dependent insulinotropic polypeptide secretion in response to nutrient ingestion in man: acute post-prandial and 24-h secretion patterns. *J Endocrinol*. 1993;138:159–166.
76. Shin ED, Estall JL, Izzo A, Drucker DJ, Brubaker PL. Mucosal adaptation to enteral nutrients is dependent on the physiologic actions of glucagon-like peptide-2 in mice. *Gastroenterology*. 2005;128:1340–1353.
77. Burrin DG, Stoll B, Jiang R, et al. GLP-2 stimulates intestinal growth in premature TPN-fed pigs by suppressing proteolysis and apoptosis. *Am J Physiol Gastrointest Liver Physiol*. 2000;279:G1249–G1256.
78. Chance WT, Sheriff S, Dayal R, Friend LA, Thomas I, Balasubramaniam A. The role of polyamines in glucagon-like peptide-2 prevention of TPN-induced gut hypoplasia. *Peptides*. 2006;27:883–892.