Soluble CD163 correlates with lipid metabolic adaptations in type 1 diabetes patients during ketoacidosis

Mads Svart1*, Nikolaj Rittig1, Niels Møller1, Holger J Møller2, Henning Gronbaek3

1Department of Internal Medicine and Endocrinology (MEA) and Medical Research Laboratory, Aarhus University Hospital, Aarhus C, 2Department of Clinical Biochemistry, Aarhus University Hospital, Aarhus N, and 3Department of Hepatology and Gastroenterology, Aarhus University, Aarhus C, Denmark

Keywords
G0/G1 switch 2 gene, Ketoacidosis, Soluble CD163

*Correspondence
Mads Svart
Tel.: +45-2811-8221
E-mail address: mvsv@clin.au.dk

J Diabetes Investig 2019; 10: 67–72
doi: 10.1111/jdi.12869

Clinical Trial Registry
ClinicalTrials.gov
NCT02157155

ABSTRACT
Introduction: Diabetic ketoacidosis (DKA) is associated with inflammation and increased lipolysis. The macrophage activation marker, soluble CD163 (sCD163), is associated with obesity, non-alcoholic fatty liver disease and type 2 diabetes. We aimed to investigate whether sCD163 correlates with key elements of lipolysis in type 1 diabetes patients during mild DKA.

Materials and Methods: We investigated nine patients with type 1 diabetes twice during: (i) euglycemic control conditions and abolus of saline; and (ii) hyperglycemic ketoacidic conditions induced by lipopolysaccharide administration combined with insulin deprivation. Blood samples, indirect calorimetry, palmitate tracer and adipose tissue biopsies were used to investigate lipid metabolism.

Results: We observed a significant increase in plasma sCD163 levels after lipopolysaccharide exposure \( P < 0.001 \). Concentrations of sCD163 were positively correlated with plasma concentrations of free fatty acids, palmitate rate of appearance and lipid oxidation rates, and negatively correlated to the expression of G0/G1 switch 2 gene messenger ribonucleic acid content in adipose tissue \( P < 0.01 \) for all. Furthermore, sCD163 levels correlated positively with plasma peak concentrations of cortisol, glucagon, tumor necrosis factor-\( \alpha \), interleukin-6 and interleukin-10 \( P < 0.01 \) for all). Data on lipolysis and inflammation have previously been published.

Conclusions: Macrophage activation assessed by sCD163 might play an important role in DKA, as it correlates strongly with important components of lipid metabolism including free fatty acids, palmitate, lipid oxidation, G0/G1 switch 2 gene and pro-inflammatory cytokines during initial steps of DKA. These results are novel and add important knowledge to the field of DKA.

INTRODUCTION
Diabetic ketoacidosis (DKA) is a life-threatening condition in patients with type 1 diabetes. The condition is characterized by accelerated lipid metabolism, resulting in the generation of ketone bodies in the liver often combined with systemic inflammation. Low-grade inflammation is a cornerstone in a broad variety of metabolic disorders; for example, obesity, non-alcoholic fatty liver disease and type 2 diabetes, and it is evident that macrophages play an important role in such conditions.1-3. Soluble CD163 (sCD163) is shed from the surface of macrophages during inflammation4. Recently, it has been shown that baseline concentrations of sCD163 in type 1 diabetes are comparable with the concentrations of sCD163 in type 2 diabetes, which in turn could indicate similar baseline macrophage activation in type 1 diabetes5,6.

Lipopolysaccharide (LPS) has long been used as a model mimicking the inflammatory response observed in septic patients.7 The LPS molecule, being the major virulence factor of Gram-negative bacteria, binds to Toll-like receptors in the cell membrane of innate immune cells (e.g., macrophages) and an intracellular signaling cascade is initiated, which eventually leads to the transcription and release of a wide range of pro-
and anti-inflammatory cytokines; for example, tumor necrosis factor-α (TNF-α)⁶⁻⁹. The same mechanisms lead to shedding of sCD163¹⁰.

Along with inflammation, altered lipid metabolism plays a critical role in metabolic disorders, including DKA, NAFLD and type 2 diabetes. Lipid droplet-associated proteins control lipolysis; for example, adipose triglyceride lipase and G0/G1 switch gene 2 (G0S2), the latter being mainly responsible for decelerating lipolysis by directly inhibiting adipose triglyceride lipase¹¹,¹². Previous studies on acute inflammation have shown that administration of TNF-α or LPS in individuals without diabetes increases circulating levels of stress hormones and stimulates lipolysis¹³,¹⁴.

The primary objective of the present study was to investigate whether plasma sCD163 correlates with changes in lipid metabolism during the initial steps of DKA in type 1 diabetes patients. We used a model combining LPS exposure and insulin deficiency to induce DKA.

METHODS
Participants and study protocol
Data originate from a previously published human randomized, controlled, cross-over trial consisting of two experimental days, separated by at least 3 weeks¹⁵ in which data on lipid metabolism and inflammatory responses have been given. In short, nine male volunteers with type 1 diabetes were selected using inclusion criteria as follows: male, aged 20–40 years, C-peptide negative, no medication other than insulin, body mass index 19–26 kg/m² and no comorbidities, including diabetic complications.

The two study days were characterized by: (i) euglycemic, non-inflammatory control conditions (CTR) with a bolus of saline (154 mmol/L NaCl); and (ii) hyperglycemic, inflammatory, ketotic conditions (KET) induced by a LPS bolus administration combined with insulin deprivation to 15% of the participants’ regular basal rates¹⁵. The time-course of the study days was 0–300 min. The study was carried out at Aarhus University Hospital, Aarhus, Denmark.

The study was carried out in accordance with the Declaration of Helsinki, and all study participants gave their written and oral informed consent to participate. The Local Ethics Committee approved the study protocol (1-10-72-98-14) and registered at www.clinicaltrials.gov (ID number: NCT02157155).

Lipid metabolism
Indirect calorimetry (Oxycon Pro; Intramedic, Gentofte, Denmark) was applied at 150 min to measure lipid oxidation rates as described by Ferrannini¹⁶.

Albumin-bound [9,10-3H]-palmitate (PerkinElmer, Mecheleen, Belgium; Department of Clinical Physiology and Nuclear Medicine, Aarhus University Hospital, Aarhus, Denmark) was infused (0.3 mCi/min) at 200–260 min and analyzed in triplicate samples using previously described calculations¹⁷.

An abdominal subcutaneous adipose tissue biopsy was obtained at 270 min. Messenger ribonucleic acid (mRNA) was isolated using TRIZol (Gibco BRL; Life Technologies, Roskilde, Denmark), and quantitative polymerase chain reaction was carried out in a LightCycler 480 (Roche Life Science, Indianapolis, IN, USA). The primer sequence used for G0S2 was 5’ CGA GAG CCC AGA GCC GAG ATG 3’ and 5’ AGC ACC AGC CCG AAG AG 3’, 137 bp. The G0S2 gene was quantified using the housekeeping gene, glyceraldehyde 3-phosphate dehydrogenase. Glyceraldehyde 3-phosphate dehydrogenase was tested and found to be similar during both conditions.

Serum concentrations of β-hydroxybutyrate (hydrophilic interaction liquid chromatography tandem mass spectrometry), free fatty acids (FFA; in vitro enzymatic colorimetric method assay NEFA-HR(2); Wako Chemicals GmbH, Neuss, Germany), serum concentrations of cortisol (enzyme-linked immunosorbent assay; DRG Diagnostic, Marburg, Germany) and glucagon (EMD, Darmstadt, Germany) were all analyzed in accordance with the manufacturer’s recommendations. All blood samples were stored at ~20°C and analyzed in the same assay after all participants had completed both days.

Inflammation
To mimic infection, an Escherichia coli endotoxin/LPS (10,000 USP Endotoxin, lot HOK354; U.S. Pharmacopeial Convention, Rockville, MD, USA) was diluted in isotonic saline and infused intravenously as a bolus of 1 ng/kg bodyweight at 0 min. Serum concentrations of TNF-α, interleukin (IL)-6 and IL-10 were measured in a magnetic Bio-Plex Pro Human Chemokine Assay (Bio-Rad, Hercules, CA, USA). An in-house assay was used to measure sCD163 (BEP-2000 enzyme-linked immunosorbent assay analyzer; Dade Behring, Marburg, Germany) essentially as previously described¹⁸. The peak increment (Δ) in plasma concentration was calculated as peak concentration – baseline concentration.

Statistical analysis
Statistical graphing and analyses were completed using Sigma-plot 11 (Systat Software, San Jose, CA, USA) and Stata 13 (StataCorp, College Station, TX, USA), respectively. P-values <0.05 were considered significant. Two-way repeated measures ANOVA was used to test for interactions in sCD163 concentrations during time × groups (CTR and KET). In the event of a significant interaction, multiple pairwise comparisons were carried out using the Student–Newman–Keuls method. Correlations were evaluated using parametric linear regression including tests to assure that the correlations were linear. Normal distribution of data was ensured by inspection of Q-Q plots.

RESULTS
All nine participants completed both study days and were included in the analyses. Clinical and biochemical characteristics have previously been published¹⁵. In brief, the participants had been diagnosed with diabetes for 14 ± 2 years. They had an
average glycated hemoglobin of 7.7% (61 mmol/mol), a body mass index of 25 ± 1 kg/m², an insulin dose of 0.7 ± 0.4 U/kg/day and a median age of 30 years (range 21–40 years).

SCD163
At the time of LPS bolus infusion, all participants had comparable concentrations of sCD163 ~1.5 mg/L (P = 0.4). However, the concentration promptly rose 2–3-fold in KET condition and remained elevated throughout the trial (P < 0.001). During CTR conditions, sCD163 did not change from baseline concentrations (Figure 1).

Lipid metabolism
The sCD163 levels correlated positively with concentrations of FFA, β-hydroxybutyrate and lipid oxidation (P < 0.01; Figure 2), and with the palmitate rate of appearance (P < 0.001; Figure 2). Furthermore, polymerase chain reaction analysis of G0S2 mRNA content from the adipose tissue biopsies showed a negative correlation with sCD163 (P < 0.001; Figure 2).

Inflammation
We found a positive correlation between ΔsCD163 and plasma cytokines ΔTNF-α, ΔIL-6 and ΔIL-10 (P < 0.001; Figure 3). Additionally, there was a strong positive correlation between plasma Δglucagon and Δcortisol (P < 0.001; Figure 3).

Figure 1 | Plasma soluble CD163 (sCD163) concentrations. The mean plasma concentration (± standard error of the mean) of sCD163 during the study period is shown for control (○) and ketotic (●) conditions. Repeated measurements two-way ANOVA analysis was used to test for differences between groups (n = 9).

Figure 2 | Lipid metabolism and soluble CD163 (sCD163). Data is presented as dot plots showing (a) plasma concentrations of free fatty acids (FFA), (b) plasma concentrations of β-hydroxybutyrate (BHB), (c) lipid oxidation rates, (d) rate of appearance for palmitate (palmitate[Rₐ]) and (e) messenger ribonucleic acid (mRNA; G0G1 switch gene 2 [G0S2] on the x-axis and concentrations of sCD163 on the y-axis after exposure to control (○) and ketotic (●) conditions). All measurements were carried out at approximately 300 min. A parametric linear regression analysis was used to test for correlations (n = 9, P < 0.01 for all).
DISCUSSION

The present study shows how sCD163 increases in type 1 diabetes patients during the early stages of DKA induced by LPS exposure and lack of insulin. We found significant positive correlations between sCD163 and central elements of lipid metabolism and cytokines during early phases of DKA combined with a negative correlation with G0S2 mRNA content in adipose tissue samples. These results bring new insight into the field of DKA, suggesting a central role for the macrophages in the lipid metabolic adaptations characterizing this condition.

In agreement with previous studies investigating sCD163 after LPS administration, we found rapidly increased sCD163 concentrations, that remained elevated throughout the study period. Lipolysis was studied in our trial by well-established techniques, including indirect calorimetry, palmitate tracer technique, blood sampling, and adipose tissue biopsies in the CTR and KET condition. We showed, in early stages of DKA, a significantly positive correlation between sCD163 and increases in lipid oxidation, palmitate rate of appearance, and FFA. This in vivo correlation was found within a short-term elevation of FFA (5 h). An in vitro study examined long-term exposure (24 h) of FFA to monocytes, and found diminished CD163 expression and consequently lower sCD163. In contrast, NAFLD patients with chronically elevated FFA had a strong positive correlation between sCD163 and the severity of NAFLD and increased FFA.

Additionally, we found a significant negative correlation between sCD163 and G0S2 mRNA expression. G0S2 is a critical node in reducing lipolysis. It is regulated primarily by adrenergic and insulin. The downregulation of G0S2 at protein and mRNA levels during catabolic conditions, such as fasting, strenuous exercise and sepsis, has previously been extensively reviewed. Recently, an in vitro study showed that omega-3 polyunsaturated fatty acids alter TNF-α-induced inhibition of G0S2 expression. Data from the present paper show a significant negative correlation between sCD163 and G0S2 in the catabolic state of acute DKA, which might suggest that macrophage activation affects G0S2 expression, most likely indirectly through cytokine production, for example, TNF-α.

One other study used the same concentration of LPS, but in healthy volunteers, and also showed a strong positive correlation between sCD163 and TNF-α. Although the concentration of TNF-α was roughly half of the concentration measured in the present study (400 pg/mL vs 800 pg/mL), we discovered only a slightly higher increase in sCD163 of approximately 3.5 mg/L vs 3.0 mg/L. Others have used higher doses of LPS, 2–4 ng/kg vs 1 ng/kg, resulting in a TNF-α response somehow similar to ours (~600 pg/mL vs ~800 pg/mL in the present

Figure 3 | Inflammatory cytokines/hormones and soluble CD163 (sCD163). Data is presented as dot plots showing peak plasma concentrations (Δ = maximal concentration – basal concentration [time, 0 min]) of (a) tumor necrosis factor (TNF)-α, (b) interleukin (IL)-6, (c) IL-10, (d) glucagon and (e) cortisol on the x-axis, and peak plasma concentrations (Δ) of sCD163 on the y-axis after exposure to control (○) and ketogenic (●) conditions. A parametric linear regression analysis was used to test for correlations (n = 9; P < 0.001 for all).
study). However, in these studies, approximately twofold higher concentrations of sCD163 were measured (3.5 mg/L vs 7.0 mg/L)\(^{10,25}\). The lower peak concentration found might to some extent be explained by the negative feedback from TNF-\(\alpha\), which is chronically elevated in type 1 diabetes, hence a down-regulation of CD163 on macrophages and less shedding in response to LPS\(^{26,27}\). Furthermore, sCD163 correlated positively with the two associated inflammatory cytokines, IL-6 and IL-10, in line with previous findings in septic patients\(^{28}\).

Stress hormones (cortisol and glucagon) were also positively correlated to sCD163. The cellular expression of CD163 is strongly upregulated by glucocorticoid, and this can lead to a protracted increase in sCD163 during inflammatory conditions\(^{29}\). In vitro, several studies have shown that sCD163 shedding results from various other stimuli than LPS; for example, oxidative stress\(^{30}\), thrombin\(^{31}\) and phorbol 12-myristate 13-acetate\(^{32}\). Our data supply new in vivo evidence that macrophages might be involved in the lipid metabolic adaptations during LPS-induced DKA in type 1 diabetes patients, as emphasized by the strong positive correlations found, and underscore sCD163 as an important biomarker in these cases.

Most of the studies elaborating on the biological role of sCD163 rely on in vitro designs. Therefore, the randomized, controlled, cross-over design of the present in vivo study examining human type 1 diabetes patients adds valuable data to the existing literature in regard to sCD163, and the study suggests that macrophage activation is involved in DKA inflammation and lipolysis. It should be borne in mind that the study and its conclusions are limited to male type 1 diabetes patients. Furthermore, due to the study design, it is not possible to dissect the contribution from LPS and insulin withdrawal separately from our data. In addition, the present study was an acute study and it is therefore not possible to exclude changes in sCD163 in a more chronic setting; for example, fasting. In such a condition, growth hormone is the dominating regulator of lipolysis, and the according correlations between sCD163 and lipolytic parameters might be different.

To conclude, macrophage activation assessed by sCD163 might play an important role in DKA, as it correlates strongly with important components of lipid metabolism, including FFA, palmitate, lipid oxidation and G0S2. The response in sCD163 and the associated correlations are similar to those found in healthy individuals after LPS exposure. Cumulatively, these original findings add information on macrophages, sCD163 and involvement of DKA in type 1 diabetes.

ACKNOWLEDGMENTS

The authors thank Annette Mengel, Lisa Buus, and Helle Hau ser Ryom for their outstanding technical support and work throughout the entire study. Furthermore, we thank the nine test subjects for participating in our trial. The study was supported by the Danish Council for Strategic Research Funding (TRAIN 092797; grant nos. 0603-00479B, 10-092797). Henning Gronbaek received funding from the NOVO Nordisk Foundation, “Savvaerksejer Jeppe Juhl og hustru Ovita Juhls mindelegat”.

DISCLOSURE

The authors declare no conflict of interest.

REFERENCES

1. Fjeldborg K, Pedersen SB, Moller HJ, et al. Intrahepatic fat content correlates with soluble CD163 in relation to weight loss induced by Roux-en-Y gastric bypass. Obesity (Silver Spring) 2015; 23: 154–161.
2. Sorensen LP, Parkner T, Sondgaard E, et al. Visceral obesity is associated with increased soluble CD163 concentration in men with type 2 diabetes mellitus. Endocr Connect 2015; 4: 27–36.
3. Kazankov K, Barrera F, Moller HJ, et al. The macrophage activation marker sCD163 is associated with morphological disease stages in patients with non-alcoholic fatty liver disease. Liver Int 2016; 36: 1549–1557.
4. Moller HJ. Soluble CD163. Scand J Clin Lab Invest 2012; 72: 1–13.
5. Llaurado G, Gonzalez-Clemente JM, Maymo-Masip E, et al. Serum levels of TWEAK and scavenger receptor CD163 in type 1 diabetes mellitus: relationship with cardiovascular risk factors. A case-control study. PLoS ONE 2012; 7: e43919.
6. Parkner T, Sorensen LP, Nielsen AR, et al. Soluble CD163: a biomarker linking macrophages and insulin resistance. Diabetologia 2012; 55: 1856–1862.
7. Andreasen AS, Krabbe KS, Krogh-Madsen R, et al. Human endotoxemia as a model of systemic inflammation. Curr Med Chem 2008; 15: 1697–1705.
8. Miyake K. Endotoxin recognition molecules, Toll-like receptor 4-MD-2. Semin Immunol 2004; 16: 11–16.
9. Fijen JW, Kobold AC, de Boer P, et al. Leukocyte activation and cytokine production during experimental human endotoxemia. Eur J Intern Med 2000; 11: 89–95.
10. Etzerodt A, Maniecki MB, Moller K, et al. Tumor necrosis factor alpha-converting enzyme (TACE/ADAM17) mediates ectodomain shedding of the scavenger receptor CD163. J Leukoc Biol 2010; 88: 1201–1205.
11. Zhang X, Heckmann BL, Campbell LE, et al. G0S2: a small giant controller of lipolysis and adipose-liver fatty acid flux. Biochem Biophys Acta 2017; 1862: 1146–1154.
12. Nielsen TS, Moller N. Adipose triglyceride lipase and G0/G1 switch gene 2: approaching proof of concept. Diabetes 2014; 63: 847–849.
13. Plomgaard P, Fischer CP, Ittelt T, et al. Tumor necrosis factor-alpha modulates human in vivo lipolysis. J Clin Endocrinol Metab 2008; 93: 543–549.
14. Buhl M, Bosnjak E, Vendelbo MH, et al. Direct effects of locally administered lipopolysaccharide on glucose, lipid, and protein metabolism in the placebo-controlled, bilaterally infused human leg. J Clin Endocrinol Metab 2013; 98: 2090–2099.
15. Svart M, Kampmann U, Voss T, et al. Combined insulin deficiency and endotoxin exposure stimulate lipid mobilization and alter adipose tissue signaling in an experimental model of ketoacidosis in subjects with type 1 diabetes: a randomized, controlled, cross over trial. Diabetes 2016; 65: 1380–1386.

16. Ferrannini E. The theoretical bases of indirect calorimetry: a review. Metabolism 1988; 37: 287–301.

17. Moller N, Jensen MD, Rizza RA, et al. Renal amino acid, fat and glucose metabolism in type 1 diabetic and non-diabetic humans: effects of acute insulin withdrawal. Diabetologia 2006; 49: 1901–1908.

18. Moller HJ, Hald K, Moestrup SK. Characterization of an enzyme-linked immunosorbent assay for soluble CD163. Scand J Clin Lab Invest 2002; 62: 293–299.

19. Bauer S, Weiss TS, Wiest R, et al. Soluble CD163 is not increased in visceral fat and steatotic liver and is even suppressed by free fatty acids in vitro. Exp Mol Pathol 2011; 91: 733–739.

20. Zhang JW, Zhao Y, Xu CF, et al. Association between serum free fatty acid levels and nonalcoholic fatty liver disease: a cross-sectional study. Sci Rep 2014; 4: 5832.

21. Kazankov K, Tordjman J, Moller HJ, et al. Macrophage activation marker soluble CD163 and non-alcoholic fatty liver disease in morbidly obese patients undergoing bariatric surgery. J Gastroenterol Hepatol 2015; 30: 1293–1300.

22. Schweiger M, Paar M, Eder C, et al. G0/G1 switch gene-2 regulates human adipocyte lipolysis by affecting activity and localization of adipose triglyceride lipase. J Lipid Res 2012; 53: 2307–2317.

23. Laiglesia LM, Lorente-Cebrian S, Lopez-Yoldi M, et al. Maresin 1 inhibits TNF-alpha-induced lipolysis and autophagy in 3T3-L1 adipocytes. J Cell Physiol 2017; 233: 2238–2246.

24. Rittig N, Svart M, Jessen N, et al. Macrophage activation marker sCD163 correlates with accelerated lipolysis following LPS exposure: a human-randomised clinical trial. Endocr Connect 2018; 7: 107–114.

25. Hintz KA, Rassias AJ, Wardwell K, et al. Endotoxin induces rapid metalloproteinase-mediated shedding followed by up-regulation of the monocyte hemoglobin scavenger receptor CD163. J Leukoc Biol 2002; 72: 711–717.

26. Buechler C, Ritter M, Orso E, et al. Regulation of scavenger receptor CD163 expression in human monocytes and macrophages by pro- and antiinflammatory stimuli. J Leukoc Biol 2000; 67: 97–103.

27. Qiao YC, Chen YL, Pan YH, et al. The change of serum tumor necrosis factor alpha in patients with type 1 diabetes mellitus: a systematic review and meta-analysis. PLoS ONE 2017; 12: e0176157.

28. Gains S, Pedersen SS, Koldkaer OG, et al. New immunological serum markers in bacteraemia: anti-inflammatory soluble CD163, but not proinflammatory high mobility group-box 1 protein, is related to prognosis. Clin Exp Immunol 2008; 151: 423–431.

29. Goldstein JI, Goldstein KA, Wardwell K, et al. Increase in plasma and surface CD163 levels in patients undergoing coronary artery bypass graft surgery. Atherosclerosis 2003; 170: 325–332.

30. Timmermann M, Hogger P. Oxidative stress and 8-iso-prostaglandin F(2alpha) induce ectodomain shedding of CD163 and release of tumor necrosis factor-alpha from human monocytes. Free Radic Biol Med 2005; 39: 98–107.

31. Chung S, Kim JE, Park S, et al. Neutrophil and monocyte activation markers have prognostic impact in disseminated intravascular coagulation: in vitro effect of thrombin on monocyte CD163 shedding. Thromb Res 2011; 127: 450–456.

32. Droste A, Sorg C, Hogger P. Shedding of CD163, a novel regulatory mechanism for a member of the scavenger receptor cysteine-rich family. Biochem Biophys Res Commun 1999; 256: 110–113.