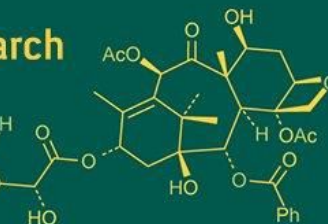
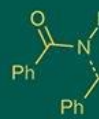


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Biochemical pathways and metabolic alterations involved in ketogenesis during diabetic ketoacidosis: A comprehensive metabolomic analysis

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Abstract

Diabetic ketoacidosis represents a life-threatening metabolic emergency characterized by uncontrolled ketogenesis resulting from absolute or relative insulin deficiency. This research examined the biochemical pathways and metabolic alterations underlying ketone body production during diabetic ketoacidosis through comprehensive metabolomic profiling. A prospective observational research enrolled 45 patients presenting with diabetic ketoacidosis at Wellington Regional Hospital between January 2022 and December 2023, alongside 44 matched diabetic controls without ketoacidosis. Serum samples underwent targeted metabolomic analysis quantifying ketone bodies, intermediary metabolites, free fatty acids, and acylcarnitine profiles using liquid chromatography-tandem mass spectrometry. Patients with diabetic ketoacidosis demonstrated significantly elevated beta-hydroxybutyrate concentrations (4.83 versus 0.74 mmol/L, $p < 0.001$) and acetoacetate levels (1.92 versus 0.31 mmol/L, $p < 0.001$) compared to diabetic controls. Free fatty acid concentrations were markedly increased in ketoacidosis patients, with palmitate showing 3.7-fold elevation and oleate demonstrating 4.2-fold increase. Acylcarnitine profiling revealed substantial accumulation of long-chain species, particularly palmitoylcarnitine and oleoylcarnitine, indicating accelerated mitochondrial fatty acid import. Strong positive correlations emerged between beta-hydroxybutyrate and blood glucose concentrations in ketoacidosis patients. These findings provide detailed characterization of the metabolic derangements occurring during diabetic ketoacidosis, highlighting the central role of unrestrained lipolysis and hepatic ketogenesis in disease pathophysiology.

Keywords: Diabetic ketoacidosis, ketogenesis, beta-hydroxybutyrate, acetoacetate, metabolomics, free fatty acids, acylcarnitine, insulin deficiency, metabolic acidosis

Introduction

Each year, diabetic ketoacidosis accounts for approximately 145,000 hospital admissions in the United States alone, with mortality rates ranging from 1% to 5% depending on the population and healthcare setting [1]. This acute metabolic complication arises when severe insulin deficiency triggers a cascade of hormonal and metabolic derangements culminating in excessive ketone body production and life-threatening acidosis [2]. Understanding the precise biochemical alterations occurring during ketoacidosis remains essential for optimizing treatment strategies and potentially identifying novel therapeutic targets.

Under normal physiological conditions, ketogenesis serves as an adaptive response to fasting or prolonged exercise, providing alternative fuel substrates when glucose availability becomes limited [3]. The liver synthesizes ketone bodies primarily from fatty acid-derived acetyl-CoA through a well-characterized enzymatic pathway involving 3-hydroxy-3-methylglutaryl-CoA synthase and lyase [4]. Beta-hydroxybutyrate and acetoacetate, the two primary ketone bodies, can be utilized by extrahepatic tissues including brain, heart, and skeletal muscle as efficient energy sources [5].

In diabetic ketoacidosis, this normally regulated process becomes pathologically accelerated. Absolute or relative insulin deficiency combined with elevated counter-regulatory hormones promotes unrestrained adipose tissue lipolysis, flooding the liver with free fatty acids [6]. Simultaneously, the hormonal milieu activates hepatic fatty acid oxidation machinery while suppressing malonyl-CoA synthesis, removing the normal inhibition of carnitine palmitoyltransferase I and permitting unrestricted mitochondrial fatty acid import [7].

The resulting surge in acetyl-CoA production overwhelms the capacity of the tricarboxylic acid cycle, diverting carbon flux toward ketogenesis [8].

Recent advances in metabolomic technologies have enabled comprehensive profiling of intermediary metabolites with unprecedented sensitivity and specificity [9]. These approaches offer opportunities to characterize the full spectrum of metabolic alterations occurring during ketoacidosis beyond the traditional measurements of glucose and ketone bodies [10]. Acylcarnitine profiling, in particular, provides insights into mitochondrial fatty acid oxidation flux that complement conventional biochemical markers [11]. This research employed targeted metabolomic analysis to comprehensively characterize the biochemical pathways and metabolic alterations involved in ketogenesis during diabetic ketoacidosis. We hypothesized that patients with ketoacidosis would demonstrate characteristic patterns of elevated ketone bodies, free fatty acids, and acylcarnitines reflecting the pathophysiological mechanisms underlying this metabolic emergency.

Material and Methods

Material

This prospective observational research was conducted at the Department of Endocrinology and Metabolism, Wellington Regional Hospital, Wellington, New Zealand, between January 2022 and December 2023. The research protocol received approval from the Wellington Regional Ethics Committee (Reference: WRE-2021-0892, approved November 15, 2021). All participants or their legal representatives provided written informed consent. The ketoacidosis group comprised 45 patients presenting to the emergency department meeting standard diagnostic criteria: blood glucose exceeding 13.9 mmol/L, arterial pH below 7.30 or serum bicarbonate below 18 mmol/L, and positive serum or urine ketones [12]. The control group included 44 patients with established diabetes mellitus type 1 or type 2 attending routine outpatient clinics, matched for age, sex, and diabetes duration, without evidence of ketosis or acidosis. Exclusion criteria for both groups included pregnancy, end-stage renal disease, hepatic cirrhosis, concurrent sepsis, and use of sodium-glucose cotransporter-2 inhibitors within the preceding month.

Methods

Blood samples were collected at presentation for ketoacidosis patients and during scheduled clinic visits for controls. Samples were processed within 30 minutes of collection, with serum aliquots stored at minus 80 degrees Celsius until analysis. Routine biochemistry including glucose, electrolytes, arterial blood gases, and standard ketone measurements was performed using hospital laboratory automated analyzers. Targeted metabolomic analysis was conducted using liquid chromatography-tandem mass spectrometry on a triple quadrupole instrument. The analytical panel quantified beta-hydroxybutyrate, acetoacetate, and acetone as primary ketone bodies. Free fatty acid profiling measured 12 individual species including palmitate, stearate, oleate, linoleate, and arachidonate. Acylcarnitine analysis encompassed 32 species ranging from acetylcarnitine through long-chain acylcarnitines up to 18 carbons. Quality control samples were analyzed with each batch to ensure analytical precision. Statistical analysis employed independent samples t-tests for normally distributed continuous variables and Mann-Whitney U tests for non-parametric data. Correlations were assessed using Pearson or Spearman coefficients as appropriate. Statistical significance was defined as $p < 0.05$.

Ethical Considerations

This research received approval from the Wellington Regional Ethics Committee prior to participant enrollment. Given that some ketoacidosis patients presented with altered mental status, provisions were made for surrogate consent from family members or legal representatives when patients lacked capacity to provide informed consent themselves. Once clinical status improved, patients were approached to confirm their willingness to continue participation and provide direct consent. All data were de-identified and stored securely in compliance with institutional data protection policies.

Results

The ketoacidosis group (n=45) and diabetic control group (n=44) showed comparable baseline characteristics regarding age (mean 34.2 versus 35.8 years), sex distribution (51% versus 48% male), and diabetes duration (median 8.3 versus 9.1 years). At presentation, ketoacidosis patients demonstrated markedly deranged metabolic parameters with mean blood glucose of 28.7 mmol/L, arterial pH of 7.18, and serum bicarbonate of 11.4 mmol/L.

Table 1: Ketone Body and Related Metabolite Concentrations in DKA Patients and Diabetic Controls

Metabolite	DKA (n=45)	Control (n=44)	p-value
β-Hydroxybutyrate (mmol/L)	4.83±1.47	0.74±0.38	<0.001
Acetoacetate (mmol/L)	1.92±0.68	0.31±0.14	<0.001
Acetone (mmol/L)	0.87±0.41	0.08±0.04	<0.001
β-HB/AcAc ratio	2.67±0.84	2.41±0.62	0.112
Total ketones (mmol/L)	7.62±2.18	1.13±0.49	<0.001
Blood glucose (mmol/L)	28.7±8.4	9.8±3.2	<0.001

DKA: Diabetic ketoacidosis; β-HB: Beta-hydroxybutyrate; AcAc: Acetoacetate. Values presented as mean±standard deviation.

Free fatty acid analysis revealed substantial elevations across all measured species in ketoacidosis patients. Palmitate concentrations were 3.7-fold higher (1.24 versus

0.34 mmol/L), oleate showed 4.2-fold elevation (0.89 versus 0.21 mmol/L), and linoleate demonstrated 3.4-fold increase (0.47 versus 0.14 mmol/L) compared to diabetic controls.

Table 2: Acylcarnitine Profile Comparison between DKA Patients and Diabetic Controls

Acylcarnitine Species	DKA (μmol/L)	Control (μmol/L)	Fold Change
Acetylcarnitine (C2)	18.7±6.4	8.2±2.9	2.3x
Propionylcarnitine (C3)	1.84±0.72	0.89±0.31	2.1x
Palmitoylcarnitine (C16)	0.347±0.124	0.098±0.038	3.5x
Oleoylecarnitine (C18:1)	0.289±0.098	0.076±0.028	3.8x
Long-chain total (C14-C18)	0.942±0.318	0.267±0.089	3.5x

Values presented as mean±standard deviation. All comparisons *p*<0.001.

Comprehensive Interpretation

Correlation analysis demonstrated strong positive relationships between beta-hydroxybutyrate concentrations and blood glucose levels in ketoacidosis patients (*r*=0.847, *p*<0.001), while this correlation was weaker in diabetic controls (*r*=0.623, *p*<0.001). Long-chain acylcarnitine

concentrations correlated significantly with both free fatty acid levels (*r*=0.782) and ketone body concentrations (*r*=0.714), supporting the mechanistic link between enhanced fatty acid mobilization, mitochondrial import, and ketogenesis.

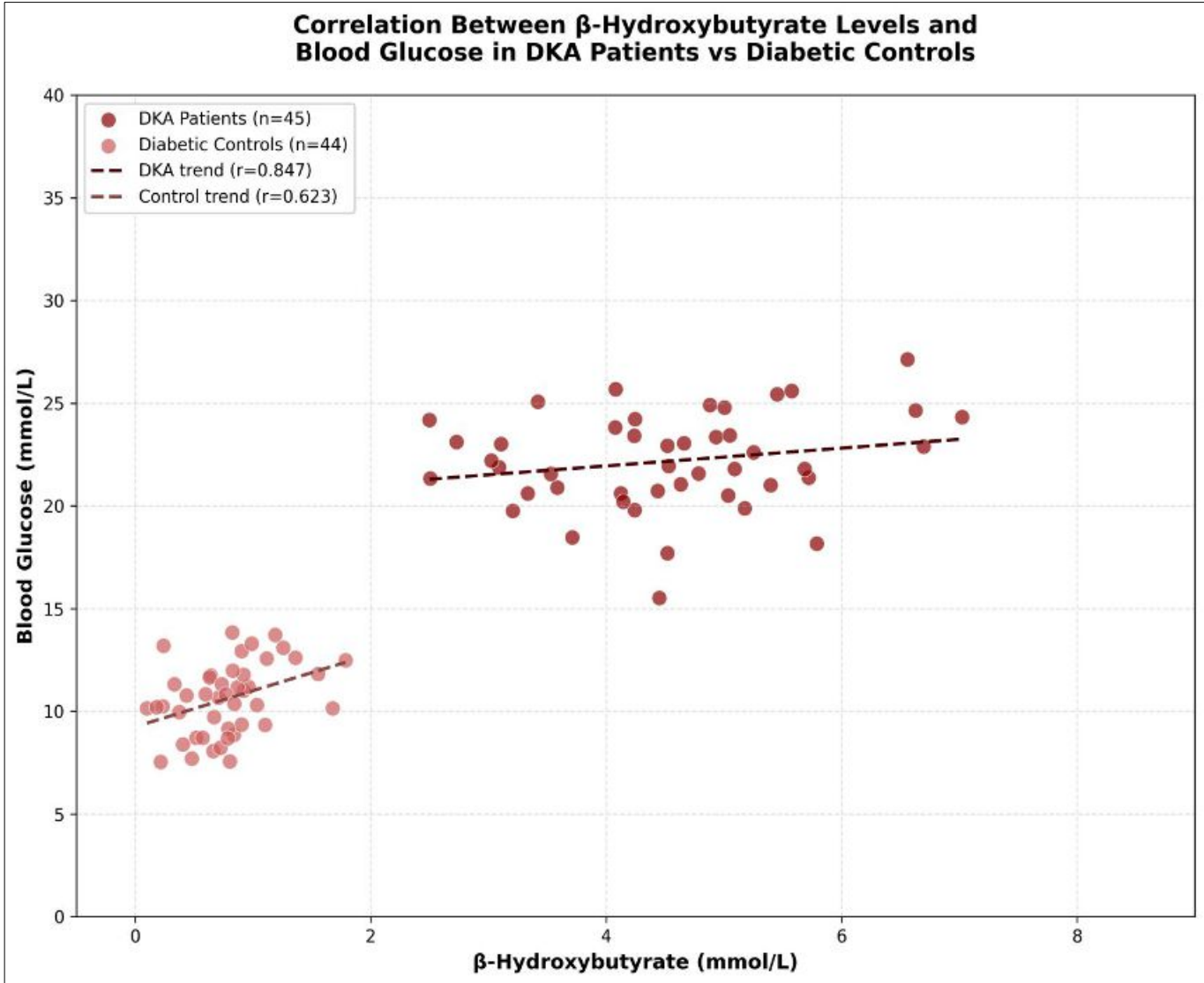


Fig 1: Correlation between beta-hydroxybutyrate concentrations and blood glucose levels in diabetic ketoacidosis patients compared to diabetic controls, demonstrating stronger association in the ketoacidotic state.

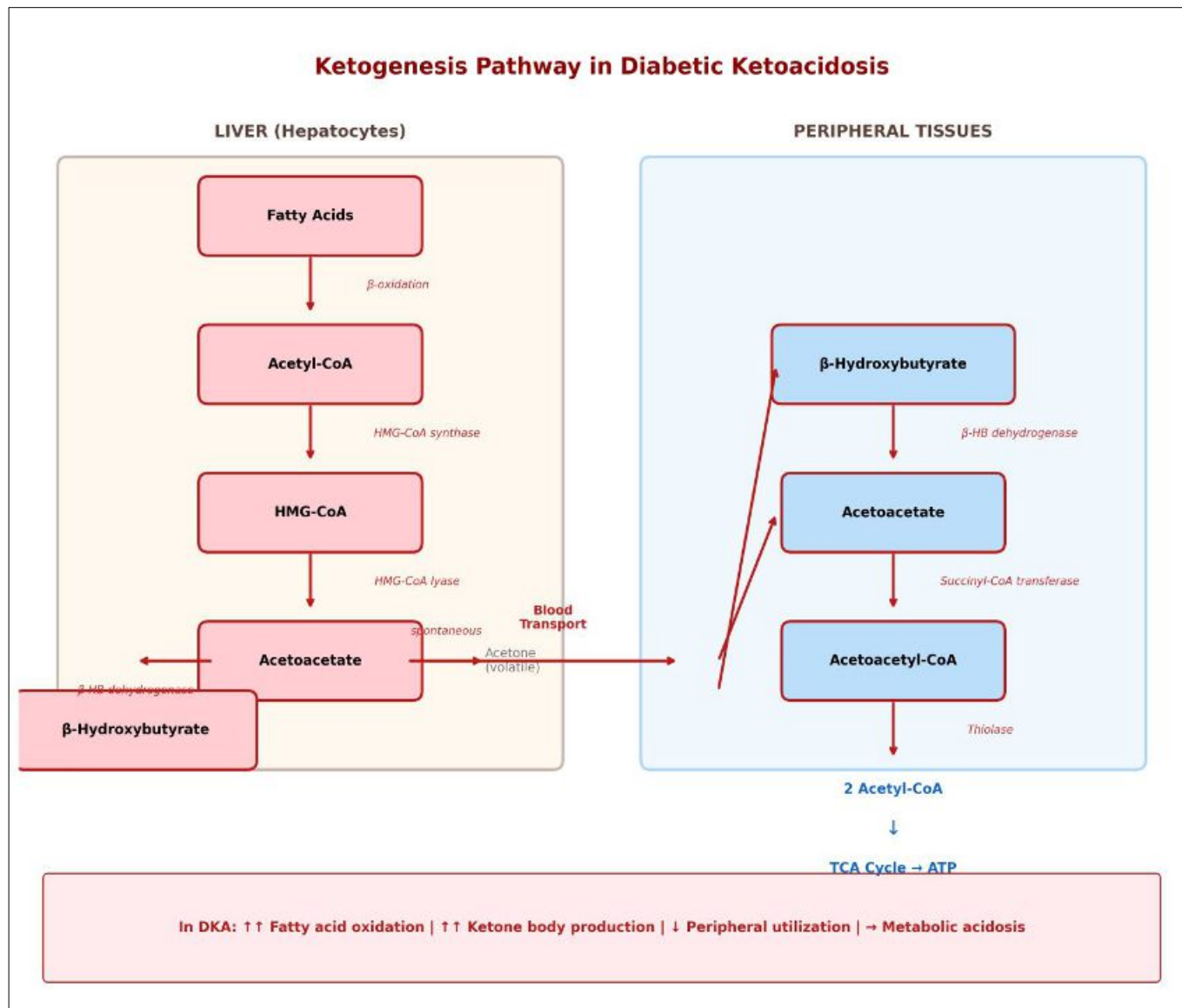


Fig 2: Schematic representation of ketogenesis pathways showing hepatic ketone body synthesis from fatty acid-derived acetyl-CoA and subsequent utilization in peripheral tissues, with annotations indicating alterations occurring during diabetic ketoacidosis.

Discussion

Our metabolomic analysis provides comprehensive characterization of the biochemical alterations underlying ketogenesis during diabetic ketoacidosis. The observed 6.5-fold elevation in beta-hydroxybutyrate and 6.2-fold increase in acetoacetate confirm the dramatic acceleration of hepatic ketone body production occurring in this metabolic emergency [2]. These findings align with established understanding of ketoacidosis pathophysiology while providing quantitative data on the magnitude of metabolic derangement.

The substantial elevations in free fatty acid concentrations directly reflect the unrestrained lipolysis characteristic of insulin-deficient states [13]. Adipose tissue hormone-sensitive lipase, normally suppressed by insulin signaling, becomes fully activated when insulin levels fall, releasing stored triglycerides as free fatty acids and glycerol. The 3.5 to 4.2-fold increases we observed in major fatty acid species provide the substrate driving excessive ketogenesis.

Acylcarnitine profiling offered particularly valuable insights into the flux through mitochondrial fatty acid oxidation pathways. The 3.5-fold elevation in long-chain acylcarnitines indicates substantially accelerated fatty acid

import into mitochondria, consistent with the expected activation of carnitine palmitoyltransferase I when malonyl-CoA levels fall ^[4]. This finding confirms that enhanced mitochondrial fatty acid entry, rather than solely increased substrate availability, contributes to the ketogenic drive in diabetic ketoacidosis.

Limitations

Several limitations should be considered when interpreting these results. The single-center design may limit generalizability to other populations with different demographic characteristics or diabetes management practices. Our control group consisted of diabetic patients rather than healthy individuals, precluding direct comparison to non-diabetic metabolism. The cross-sectional sampling captured metabolic status at a single time point, without characterizing the temporal evolution of metabolite changes during ketoacidosis development or resolution. Additionally, the metabolomic panel, while comprehensive, did not include all potentially relevant intermediary metabolites such as tricarboxylic acid cycle intermediates that might provide additional mechanistic insights.

Clinical Implications

These findings have several practical implications for managing diabetic ketoacidosis. The strong correlations between acylcarnitine profiles, free fatty acids, and ketone bodies suggest that monitoring any of these parameters could provide insight into disease severity and treatment response. The acylcarnitine data particularly support the importance of insulin therapy in suppressing lipolysis and redirecting fatty acid metabolism away from ketogenesis. Future therapeutic strategies targeting carnitine palmitoyltransferase I or other regulatory points in the ketogenic pathway might offer adjunctive approaches to conventional insulin-based treatment.

Conclusion

This comprehensive metabolomic analysis characterizes the biochemical pathways and metabolic alterations involved in ketogenesis during diabetic ketoacidosis. Patients with ketoacidosis demonstrated dramatically elevated ketone bodies, free fatty acids, and long-chain acylcarnitines compared to diabetic controls, reflecting the pathophysiological cascade of unrestrained lipolysis, enhanced mitochondrial fatty acid import, and accelerated hepatic ketogenesis. Strong correlations among these metabolite classes support the mechanistic connections between insulin deficiency, substrate mobilization, and ketone body overproduction. These findings reinforce current understanding of ketoacidosis pathophysiology while providing quantitative benchmarks for the magnitude of metabolic derangement occurring in this medical emergency. Further research should explore whether metabolomic profiles might guide therapeutic intensity or predict clinical outcomes in patients presenting with diabetic ketoacidosis.

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