

# The Impact of Different BHB Substrates and Precursors on Liver ATP

## ABSTRACT

This study investigated the impact of various beta-hydroxybutyrate (BHB) substances and BHB precursors (BHB sources) on liver ATP (adenosine triphosphate) levels. We administered L-BHB, D-BHB, BHP-Na, BKP-Na, 1,3-butanediol, C8 MCT (medium-chain triglycerides), D-BHB + C8 MCT, and related esters to a mouse model, measuring liver ATP production at 4-time intervals over 120 minutes.

Our results showed distinct differences among the BHB sources. D-BHB + C8 MCT, D-BHB, L-BHB, and C8 MCT increased liver ATP, with D-BHB + C8 MCT yielding the highest increase. Conversely, 1,3-butanediol, and 1,3-butanediol ester derivatives, significantly depleted liver ATP due to their energy-intensive metabolic conversion process into BHB.

## BACKGROUND

The recent surge of interest in beta-hydroxybutyrate (BHB) and its precursors (BHB sources) as potential metabolic fuels necessitates a deeper understanding of their true ATP energy impact. While blood ketone measurements are valuable for tracking ketone blood levels and the state of ketosis (blood ketone levels over 0.5mmol/l), they offer a limited perspective on total cellular energy (ATP).

BHB substrates and precursors (BHB sources) undergo various metabolic conversions in the liver before becoming bioavailable as ketone bodies (BHB), each with a unique ATP energy cost profile. By emphasizing "net" ATP generation of BHB sources through these metabolic processes, we can paint a more comprehensive picture of their actual metabolic efficiency beyond ketosis.

### Why This Distinction Matters:

- Substrate Energetics:** Not all BHB sources are created equal. Some may require more ATP investment during their conversion process compared to others. Consequently, even if two BHB sources raise blood BHB levels comparably, the net ATP yield could differ significantly.

- **Cellular Fueling:** Cellular energy demands are not met by BHB alone. The "net" ATP generated during the conversion of BHB sources ultimately determines how efficiently a BHB source replenishes cellular energy stores. Understanding this production process helps identify which BHB sources offer the most favorable balance between BHB elevation in ketosis and the cellular energy costs of conversion.
- **Beyond Ketosis:** While measuring blood ketones is helpful for confirming ketosis, it doesn't tell the whole story about how efficiently your body uses BHB for energy (ATP). Focusing solely on blood ketone levels overlooks the complex steps involved in converting BHB into usable energy and the overall energy produced by BHB within cells (ketolysis).
- **Therapeutic Potential:** In therapeutic contexts where energy support is paramount (such as metabolic disorders or neurodegenerative conditions), deciphering the net ATP yield of various BHB sources becomes crucial. It guides us towards understanding which BHB sources most effectively fuel cellular functions under different circumstances and how BHB sources might be paired together to create even better applications, rather than simply promoting BHB sources based on ketone level elevation alone.

## STUDY OBJECTIVE

This study investigates the often-overlooked metabolic costs associated with different BHB sources, focusing on liver ATP generation during their conversion into bioavailable BHB. Our goal is to provide a more comprehensive understanding of BHB source efficiency and identify metrics beyond blood ketone levels.

## STUDY DESIGN

### Methods:

We examined the effects of eight different BHB sources proven to increase BHB levels in the blood. ATP was measured at various time points post administration of equivalent gram dosages of each BHB substance.

## Subjects:

6-8 weeks-old male ICR mice were obtained from Nanjing Wukong Biotechnology Co. LTD. Animals were fed standard laboratory chow and had access to water ad libitum. Animals were housed in pairs (4 mice per cage) in a pathogen-free animal room under controlled conditions (12-hour light/dark cycle, 25°C). Animal use protocols were approved by the Institutional Animal Care and Use Committee (IACUC), and all National Institute of Health guidelines for the care and use of animals were followed. 6-8 mice were used at each time point of 0 minutes, 15 minutes, 30 minutes, and 120 minutes (24 mice total) for each BHB substance tested.

## About NNB Labs:

This study was conducted at NNB Labs. NNB is one of the world's leading companies and authorities on BHB sources and has the expertise and capabilities to manufacture any form of BHB source in this study. NNB is credentialed with NSF GMP, ISO 9001, FSSC 22000, ORGANIC NOP, ORGANIC EOS, KOSHER, HALAL, AND SMETA 4P.

## STUDY METHODOLOGY

### BHB Sources Investigated:

L-BHB acid, D-BHB acid, BHP-Na, BKP-Na, 1,3-butanediol, C8 MCT, D-BHB acid + C8 MCT, BHB-1,3-butanediol monoester, BHB-1,3-butanediol diester, and 1,3-butanediol C8 monoester.

### ATP Measurement Technique:

- 50-100mg liver tissue sample was used to extract ATP. Added 500-1000uL of acid to extract proportionally (1:10 = tissue: acid extracting solution) the ATP in the liver tissue from different time points. Extraction was ground in homogenizer, and centrifuged at 8000 gravity at 4°C for 10 minutes into a supernatant.
- The supernatant plus an equal amount of alkaline extracting solution, used to neutralize the supernatant, was then centrifuged at 8000 gravity at 4°C for 10 minutes to create the supernatant for performing ATP measurement.
- 10uL of the supernatant was added to a 96-well plate, 40uL of creatine kinase and creatine mixture was added to the supernatant, and after incubation at 37°C for 30 minutes, 200uL of phosphomolybdate chromogenic agent was added to the

supernatant, and then the supernatant was incubated at 37°C for 20 minutes. Light absorption values of each hole in the 96-well plate were determined at 700nm.

- The ATP detection pathway used to detect ATP was done through creatine kinase, catalyzing the reaction of creatine and ATP to produce creatine phosphate. The content of creatine phosphate was then detected by phosphomolybdate colorimetry at 700nm to determine the exact content of ATP extraction.
- Control Conditions: D-BHB acid was used as the control group, and all other groups were compared with D-BHB acid.

### Experiment Design:

Mice were intragastrically gavaged for each BHB substance at a volume of 0.1 mL/10g body weight. The intragastric doses were listed in Table 1. After gavage, we took 50-100mg liver from mice in homogenate tubes with steel balls at time intervals of 0, 15, 30, 120 minutes for ATP content testing.

**Table 1. Grouping methods of the test using the same mg dosage**

Group	BHB Source	Molecular Weight (g/mol)	Mice Dose (mmol/kg)	Mice Dose (mg/kg)	Human Daily Dose (g)
1	1,3-butanediol	90.12	18.02	1624	12.5
2	BHP-Na	140.13	11.6	1624	12.5
3	BKP-Na	138.11	11.8	1624	12.5
4	D-BHB acid	104.11	15.6	1624	12.5
5	C8 MCT	470.68	3.5	1624	12.5
6	L-BHB acid	104.11	15.6	1624	12.5
7	80% D-BHB acid + 20% C8 MCT	177.43	13.17	1624	12.5
8	BHB-1,3-butanediol monoester	176.21	9.22	1624	12.5
9	BHB-1,3-butanediol diester	262.3	6.19	1624	12.5
10	1,3-butanediol C8 monoester	342.52	4.74	1624	12.5

## STUDY RESULTS AND ANALYSIS

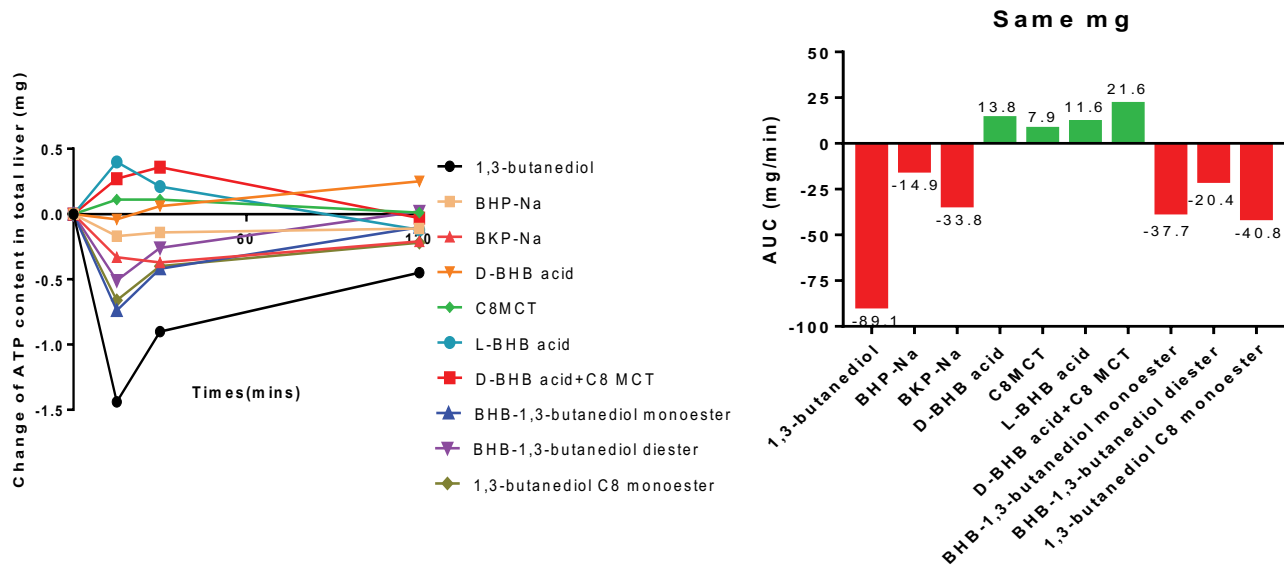


Figure 1: Changes of ATP Content in Liver and Area Under the Curve (AUC) After Gavage with Different Ketogenic Sources in the Same mg within 2 Hours

The study data reveals two distinct groups of BHB sources:

1. **ATP Generators:** D-BHB + C8 MCT, D-BHB, L-BHB, and C8 MCT demonstrated a positive net ATP impact within the liver. Among these, D-BHB + C8 MCT showed the highest ATP yield.
2. **ATP Consumers:** Every ester and 1,3-butanediol exhibited a negative net ATP impact due to their complex, ATP-demanding metabolic conversion.

### ATP Energy Trends (Generators and Consumers)

Figure 1 shows a definite trend grouping of substrates. The grouping of D-BHB + C8 MCT, D-BHB, L-BHB, and C8 all demonstrated a positive ATP Energy generative response.

The grouping of BHP-Na, BHB-1,3-butanediol diester, BKP-Na, BHB-1,3-butanediol monoester, 1,3-butanediol C8 monoester, and 1,3-butanediol demonstrated a negative ATP Energy consumption response.

D-BHB + C8 MCT demonstrates the highest level of change of ATP content in the liver, while 1,3-butanediol demonstrates the lowest.

## Net ATP Energy (Area Under the Curve)

In Figure 1, this represents the ATP generated or consumed by each substance. The AUC (area data under the curve) shown in Figure 1 occurred when an equivalent human dosage of 12.5 grams per day was given for all BHB sources.

D-BHB created 13.8 mg/min of ATP as the control BHB source in our study. 1,3-butanediol consumed 89.1 mg/min of ATP (a -755% difference from D-BHB) and D-BHB + C8 MCT generated 21.6 mg/min of ATP (a 56.5% difference from D-BHB).

## Net ATP Impact through Ketogenesis

From Figure 1, using the same gram dosage, we find that the order of net ATP production efficiency is:

1. D-BHB acid + C8 MCT
2. D-BHB acid
3. L-BHB acid
4. C8 MCT
5. BHP-Na
6. BHB-1,3-butanediol diester
7. BKP-Na
8. BHB-1,3-butanediol monoester
9. 1,3-butanediol C8 monoester
10. 1,3-butanediol

## STUDY DISCUSSION

Our findings demonstrate two distinct metabolic profiles among the investigated BHB sources, based on their net impact on liver ATP over 120 minutes. We designate them as:

### Group 1: ATP Generators

- **D-BHB + C8 MCT: Synergistic Energy** - This combination elicited the most significant ATP increase in the liver, peaking at 30 minutes followed by a gradual decline. This suggests a dual-benefit mechanism where D-BHB acid sustains energy release while C8 MCT likely fuels rapid ketone production, providing an immediate energy boost. This combination highlights the potential of strategically pairing BHB sources for optimal ATP generation.
- **D-BHB acid: Steady Direct ATP Supply** - D-BHB acid, the primary physiological ketone, exhibited a gradual ATP increase over time. As it requires no metabolic conversion, it likely provides a sustained energy source directly to liver cells.
- **L-BHB: Rapid ATP** - L-BHB showed a sharp ATP rise followed by a decline, potentially indicating rapid cellular uptake and utilization. Further research is needed to understand if this reflects specific tissue or cellular preferences for L-BHB.
- **C8 MCT: Efficient and Versatile** - C8 MCT increased liver ATP, though less dramatically than the combination of D-BHB + C8 MCT, D-BHB or L-BHB. Its conversion to acetyl-CoA drives cellular respiration (citric acid cycle) within the liver, while a portion fuels ketone generation for broader cellular energy supply.

### Group 2: ATP Consumers

- **1,3-Butanediol: Energetic Burden** - 1,3-butanediol caused the most severe ATP depletion, followed by partial recovery. Its multi-step conversion to BHB is highly ATP-demanding. This highlights a critical trade-off: while 1,3-butanediol might elevate blood ketones, it severely taxes liver energy reserves in the process, potentially outweighing any benefits.
- **1,3 Butanediol Esters: Significant ATP Cost** - BHB-1,3-butanediol esters also showed negative ATP trends, though milder than 1,3-butanediol. This confirms that including 1,3-butanediol in a BHB source, even partially, compromises the net ATP yield.

- **BHP & BKP: Mitigated ATP Cost** - These sources exhibited a less pronounced ATP decline. Their conversion pathways likely require a smaller initial ATP investment compared to 1,3-butanediol and its derivatives. However, the trend emphasizes the importance of considering metabolic costs for all BHB sources.

## CONCLUSION

This study reveals a critical distinction between BHB sources with potentially significant implications for energy metabolism and therapeutic applications. Our investigation highlights two contrasting metabolic profiles:

- **Group 1: ATP Generators:** D-BHB acid + C8 MCT, D-BHB acid, L-BHB acid, and C8 MCT demonstrated a positive net impact on liver ATP. The combination of D-BHB acid + C8 MCT proved most potent, offering both rapid and sustained energy support.
- **Group 2: ATP Consumers:** 1,3-butanediol and its related esters exhibited a negative net ATP impact due to their energy-intensive metabolic conversion processes. They raise blood ketones but at a significant cost to cellular energy reserves.

## KEY STUDY TAKEAWAYS

- **Need to Focus Beyond Ketones:** Measuring blood ketones alone fails to capture the true ATP yield and metabolic efficiency of a BHB source. Considering the metabolic 'costs' within the liver is essential for evaluating the overall energy benefits of any BHB source.
- **Importance of Metabolic Profiling:** Understanding the metabolic pathways and ATP conversion requirements of different BHB sources provides a more comprehensive picture. This conversion knowledge can be critical for tailoring BHB source selection to achieve specific metabolic goals in health and performance contexts.
- **Using Strategic Combinations:** Synergistic pairings like D-BHB acid + C8 MCT demonstrate the potential for optimizing ATP generation by providing both immediate and sustained energy support.
- **ATP: The Cellular Fuel:** This study emphasizes the need to understand the fundamental links and pathways between BHB sources and ATP. C8 MCT's fast, direct pathway provides rapid energy, while combinations like D-BHB + C8 MCT ensure continued ATP supply for various cellular functions.



- **Therapeutic Potential:** BHB sources hold promise for treating metabolic diseases, neurodegenerative conditions, myopathies, and other energy-compromised states. Understanding how ATP-generating and conversely, ATP-depleting sources figure into these contexts is crucial.

## FUTURE DIRECTIONS

- **Long-term Studies:** Research should investigate the long-term effects of BHB source combinations on cellular energy metabolism and explore their potential in managing both non-chronic and chronic conditions.
- **Human Studies:** Translating these findings to human applications of BHB sources is crucial for determining optimal dosages and protocols for specific health scenarios.
- **Targeted Applications:** Focused research on developing synergistic ATP-generating combinations like D-BHB acid + C8 MCT for brain energy support (dementia, epilepsy) and enhancing muscle function (myopathies, exercise performance) offers exciting possibilities.

**In conclusion, this study underscores the importance of going beyond simplistic blood ketone measurements in considering the net ATP yield of administering various BHB sources. This understanding paves the way for further optimizing their use in both health and performance settings.**