

# Methionine Dependence of Hair Maintenance in C57BL/6 Mice

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**Abstract.** *Background/Aim:* Hair-follicle keratinocytes contain high levels of cysteine, which is derived from methionine, rapidly proliferate, and form the hair shaft. The high proliferation rate of hair-follicle keratinocytes resembles that of aggressive cancer cells. In the present study, we determined the effect of a methionine-deficient diet on hair loss (alopecia) in mice with or without homocysteine supplementation. *Materials and Methods:* Mice were fed a normal rodent diet (2020X, ENVIGO) (Group 1); a methionine-choline-deficient diet (TD.90262, ENVIGO) (Group 2); a methionine-choline-deficient diet with a 10 mg/kg/day supply of homocysteine administered by intra-peritoneal (*i.p.*) injection for 2 weeks (Group 3). In Group 2, mice were fed a methionine-choline-deficient diet for an additional 2 weeks but with 10 mg/kg/day of *i.p.* *l*-homocysteine and the mice were observed for two additional weeks. Subsequently, the mice were fed a standard diet that included methionine. Hair loss was monitored by photography. *Results:* After 14 days, hair loss was observed in Group 2 mice on a methionine-restricted diet but not in Group 3 mice on the methionine-restricted diet which received *i.p.* homocysteine. In Group 2, at 2 weeks after methionine restriction, hair loss was not rescued by homocysteine supplementation. However, after restoration of methionine in the diet, hair growth resumed. Thus, after 2 weeks of methionine restriction, only methionine restored hair loss, not homocysteine. *Conclusion:* Hair maintenance requires

methionine in the diet. Future experiments will determine the effects of methionine restriction on hair-follicle stem cells.

In mammals, hair growth is cyclical with an anagen growth phase, catagen cessation of hair growth and a long telogen phase with loss of the hair shaft (1-4). Permanent hair loss or alopecia occurs during aging in males and females, but much more frequently in males (5, 6). In alopecia the hair follicle miniaturizes and produces a very small vellus hair shaft (7). Severe hair loss also occurs due to cancer chemotherapy in both males and females.

Cancer cells of all types arrest or lose viability by methionine restriction due to methionine addiction for transmethylation reactions (8-13).

Hair-follicle-keratinocyte proliferation is among the most rapid in the mammalian body (14). Keratinocytes form the hair shaft (4, 15, 16). The loss of rapidly proliferating keratinocytes accounts for hair loss during cytotoxic chemotherapy, which targets rapidly-cycling cells. The present report describes the effect of methionine restriction on hair maintenance in young C57BL/6 mice.

## Materials and Methods

*Mice.* C57BL/6 mice (AntiCancer Inc., San Diego, CA, USA) aged 4-6 weeks were used in the present study. The mice were housed in a controlled environment facility equipped with a High Efficiency Particulate Air (HEPA)-filtered rack, adhering to standard light/dark cycles of 12 h. The ethical committee of the AntiCancer Institutional Animal Care and Use Committee approved the use of animals, in accordance with the National Institutes of Health Guide. The studies were conducted using the Animal Research Reporting of In Vivo studies (ARRIVE) 2.0 standards.

*Diets.* During the experimental period, three groups of 5 mice each were subjected to different dietary conditions. Group 1 received a standard rodent diet (2020X, ENVIGO, Indianapolis, IN, USA); Group 2 was fed a methionine-choline-deficient diet (TD.90262, ENVIGO); and Group 3 was also fed a methionine-choline-deficient diet but received intra-peritoneal (*i.p.*) injection of *l*-homocysteine at a dose of 10 mg/kg/day. This dietary intervention was maintained

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**Key Words:** Hair loss, alopecia, methionine, homocysteine, methionine restriction.



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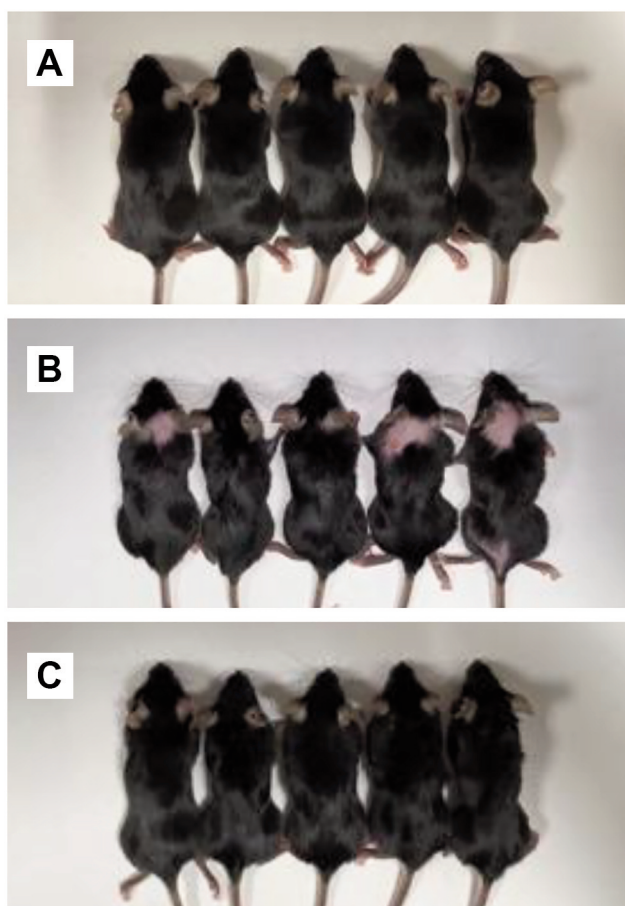


Figure 1. Mouse photos in each group after 2 weeks of treatments. A) Group 1; normal diet. B) Group 2; methionine-deficient diet for 2 weeks. C) Group 3; methionine-deficient diet with *i.p.* homocysteine supply for 2 weeks.

for a duration of two weeks. In Group 2, mice continued a methionine-choline-deficient diet for another 2 weeks but with *l*-homocysteine administered *i.p.* at 10 mg/kg/day. The effects on the dorsal mouse hair were monitored for an additional two-week period. Subsequently, the mice were reintroduced to a standard diet that included methionine.

**Blood collection.** Mouse blood samples were collected through the tail vein between the hours of 9 and 10 AM on day 15.

**Statistics.** GraphPad Prism 9.4.0 (GraphPad Software, Inc., San Diego, CA, USA) was used to conduct the statistical analyses. All data are presented as the mean and standard deviation. The level of significance was set at 0.05 or lower.

## Results

**Effect of two-week dietary methionine restriction on hair loss in C57BL/6 mice.** Mice on the methionine-containing diet in Group 1 had normal hair (Figure 1A). After 14 days on

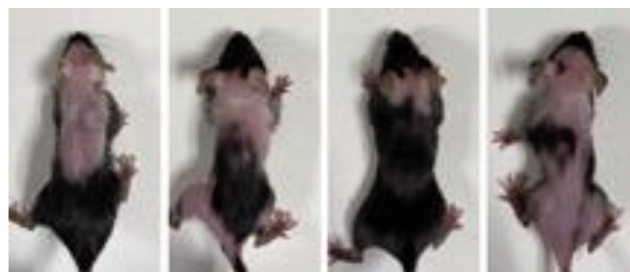


Figure 2. Mouse photo of Group 2 after 4 weeks of methionine restriction.

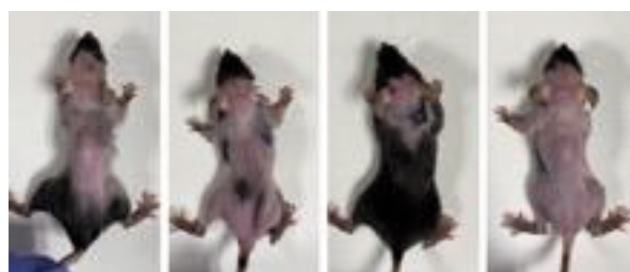


Figure 3. Mouse photo of Group 2, on a methionine-restricted diet two weeks after supplying homocysteine, which began two weeks after the start of methionine restriction.

methionine restriction, hair loss was observed in Group 2 mice (Figure 1B). Hair loss was avoided by supplying homocysteine *i.p.* to mice on the methionine-restricted diet in Group 3 (Figure 1C). In Group 2, after 4 weeks of continued methionine restriction, hair loss increased (Figure 2).

**Rescue of hair loss after methionine restriction.** After two weeks of methionine restriction, hair growth in C57BL/6 mice could not be rescued by homocysteine (Figure 3). After two weeks of methionine restriction a methionine-containing diet rescued hair growth (Figure 4).

**Blood methionine after methionine restriction.** Two weeks after methionine restriction, blood methionine decreased 48.0%, and decreased 55.2% during which homocysteine replaced methionine (Figure 5).

## Discussion

The present report demonstrates that hair maintenance in C57BL/6 mice is methionine dependent, similar to cancer cells. Homocysteine administered *i.p.*, in place of methionine in the diet, rescued hair growth in the mice during methionine restriction for 2 weeks. After 2 weeks of methionine restriction hair growth was rescued only by

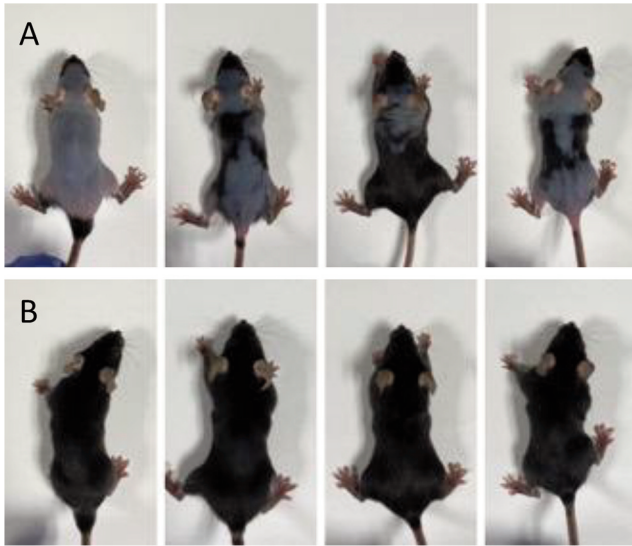


Figure 4. Photos of group 2 mice after re-introducing a normal diet containing methionine. A) Normal methionine-containing diet introduced after 1 week of methionine restriction. B) Normal methionine-containing diet introduced after 2 weeks of methionine restriction.

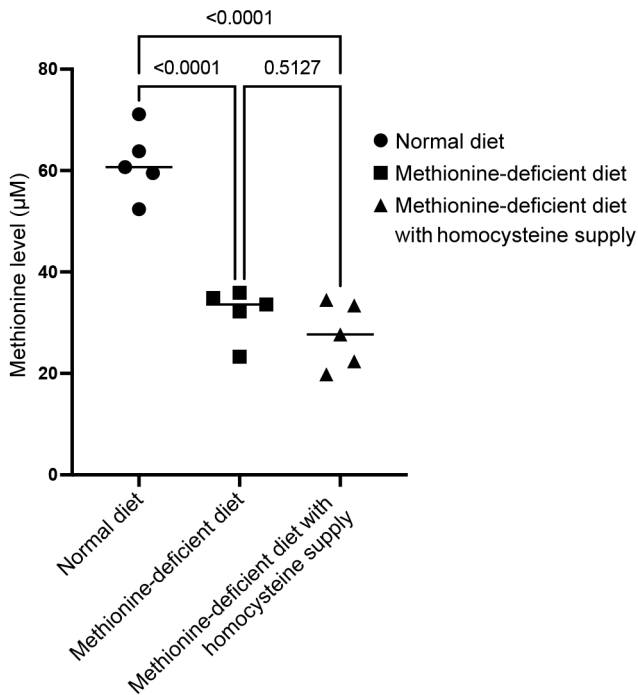


Figure 5. Blood methionine level of each group on day 15.

methionine and not by *i.p.* injection of homocysteine, which could prevent hair loss during the first 2 weeks of methionine restriction. Homocysteine did not raise the blood level of methionine and therefore could possibly directly rescue hair growth during the first 2 weeks of methionine

restriction, but not after longer periods of methionine restriction. It appears that after two weeks of methionine restriction an irreversible step occurs after which homocysteine cannot rescue hair growth. The mechanism of homocysteine rescue of hair growth during early stages of methionine restriction needs to be investigated. Homocysteine is derived from methionine and is converted to cysteine (17) and the keratinocytes of the hair shaft require a high level of cysteine.

Future experiments will investigate if hair maintenance in the mouse requires high levels of methionine in hair-follicle keratinocyte stem cells (4) or nestin expressing hair-follicle associated pluripotent (HAP) stem cells (15, 16). The effect of methionine restriction will also be investigated on the different follicle cycle stages, anagen, catagen, and telogen (2-4). Future experiments will also determine if a high-methionine diet can reverse age-related alopecia.

## Conflicts of Interest

The Authors declare no competing interests regarding this work.

## Authors' Contributions

YK, NV, and KK performed experiments. YK, and RMH wrote the article. TT provided critical suggestions.

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