Gray Hair
Gray hair => increases the risk of myocardial infarction

Figure: Men with gray hair had an increased risk of myocardial infarction. A similar although weaker and not statistically significant trend was seen in women. n = 20,000 men and women (Copenhagen City Heart Study). During the 12-year follow-up, 750 cases of first-time myocardial infarction were observed.


Epidemiological Research Unit, Rigshospitalet, Denmark
The absence of gray hair => reduces mortality in men,
The presence of arcus senilis => increases it in women

Figure: The absence of gray hair significantly reduces the risk of dying in men, while the presence of arcus senilis increases it in women; otherwise the varying degrees of graying of the hair, baldness or of facial wrinkling did not change the risk.

n = 20,000 men and women (Copenhagen City Heart Study); During 16 years of follow-up, 3,939 persons (1,656 women and 2,283 men) had died.

MSH

Against

Gray Hair?
MSH Deficiency??

Grey hair
Melanogenesis in hair

The melanoblasts => differentiate into melanocytes in skin & at the base of each hair => produce melanin pigments particles = granules => incorporated into the growing hair

Cross-section of a hair follicle
Light-haired humans

$=> \downarrow \alpha$-MSH & ACTH

**SUBJECTS:** 40 young male volunteers + different pigmentation levels (Caucasians: skin types I, II, III. Blacks: skin type VI).

**REPORTS:**

- Sign. $\downarrow$ mean basal alpha-MSH & ACTH in light-haired persons in comparison with dark-haired and black persons ($p < 0.05$)
- Sign. $\uparrow$ alpha-MSH & ACTH (alpha-MSH: skin type I: 26.7%, skin type II: 22.7%) in persons less pigmented within a short time after UV A irradiation of the whole body vs more pigmented subjects

Senile white Hair => unresponsive to MSH, because of absence of binding sites

MSH-binding sites => only in melanocytes

MATERIAL: Human hair of different colors, ranging from black to blond and senile white. => alpha-MSH binding assay + alpha-MSH analogue; controls of each assay, fragments of hairs of the same person

RESULTS: => dose-response assoc. & the assay seems to be specific for alpha-MSH, because other peptides such as ACTH, beta-LPH and beta-endorphins do not compete for binding sites as alpha-MSH does.

These binding sites seem to be present only on melanin synthesizing melanocytes, since the controls & follicles of senile white hair, which do not contain active melanocytes, show negative results.

=> absence was demonstrated on senile white hair follicles.

Red Hair => unresponsive to MSH

In vitro, human epidermal melanocytes respond to MSH -> increases in melanogenesis & cell dendricity. However, in the present study, not all melanocyte cultures exhibited these changes & 24% were totally unresponsive to MSH. This unresponsiveness was limited to melanocytes from white Europeans but was particularly prevalent in cultures from individuals with red hair.

Cyclic AMP, the second messenger of the melanocyte-associated MSH (MC-1) receptor, induced melanocyte dendricity, even in those cultures which were morphologically unresponsive to MSH. The cyclic nucleotide also increased melanogenesis in the cultures that responded melanogenically to MSH but its effect was reduced in those cultures that were unresponsive to MSH. Thus, while the morphological data support the hypothesis that unresponsiveness is mediated at the MSH receptor, intracellular events may also be important in determining melanogenic unresponsiveness to MSH.

Melanocytes of Red hair individuals

=> 0 or ↓↓ response to Alpha-MSH

- Red hair = pale skin in individuals => associated + UV-radiation sensitivity & increased skin cancer risk
- Red hair = pale skin => mainly due to polymorphisms in the melanocortin-1 receptor (MC1R) expressed in melanocytes
- MSH treatment => negligible or a much reduced response to MSH in melanocytes expressing MC1R red hair color (RHC) variant alleles.

Roberts DW, Newton RA, Leonard JH, Sturm RA. Melanocytes expressing MC1R polymorphisms associated with red hair color have altered MSH-ligand activated pigmentary responses in coculture with keratinocytes. J Cell Physiol. 2008 May;215(2):344-55. Melanogenix Group, Institute for Molecular Bioscience, University of Queensland, Brisbane, Queensland, Australia
Red hair, fair skin, low tanning => MC1R variant

Polymorphism at only 1 locus, the melanocortin-1 receptor (MC1-R), => associated with physiologic variation in hair & skin color. The MC1-R, a G protein-coupled receptor with 7 transmembrane-spanning domains, => key role in determining the type of melanin (eumelanin vs pheomelanin) that is produced within melanocytes.

- Human MC1-R variants => approximately 50% of white populations.
- Increasing evidence that loss-of-function MC1-R mutations largely account for the red hair phenotype in humans (which approximates an autosomal recessive trait) & also have a strong association with fair skin & a decreased ability to tan, with a significant heterozygote effect in individuals without red hair.
- Loss-of-function MC1-R variants may increase the risk of developing melanoma & nonmelanoma skin cancer beyond their effects on pigmentation phenotype.

Schaffer JV, Bolognia JL.. The melanocortin-1 receptor: red hair and beyond. Arch Dermatol. 2001 Nov;137(11):1477-85. Department of Dermatology, Yale University School of Medicine, 333 Cedar St, New Haven, CT 06520, USA. votavajr@biomed.med.yale.edu
ASHY DEER MICE (Peromyscus maniculatus) => become progressively grayer with subsequent molts. The trait is inherited as an autosomal recessive & the symbol ashy is assigned for the locus. The trait is distinctly manifest by 6 months of age, at which time homozygotes have white hairs on the muzzle & at the base of the tail. The amount of white gradually increases with age, but development varies greatly among animals. Some become virtually all white by 18 months.

TREATMENT: Implants of MSH => production of pigment in depigmented portions of the coat, indicating that viable melanocytes were present...

Figure 3. Semiquantitative analysis of IR changes for key HPA axis elements in organ-cultured human hair follicles. The selected, standardized reference areas indicated in Figure 2 were analyzed in the ORS by NIH image software. (Student’s t test; *P<0.05, indicating significant differences to the vehicle control). ACTH-IR in ORS (A), α-MSH in ORS (B), CRHR1/2-IR in ORS (C), MC1R-IR in ORS (D), MC2R-IR in ORS (E), cortisol-IR in ORS (F), GR-IR in ORS (G), and CRHRIR (H) in basement membrane are shown. A–C) IR of CRH-R1/2 and POMC-derived peptides in ORS are up-regulated by CRH treatment. D, E) CRH treatment modulate melanocortin receptors (MC1-R, MC2-R) in ORS. F) ACTH

Ito N, Ito T, Kromminga A, Bettermann A, Takigawa M, Kees F, Straub RH, Paus R. Human hair follicles display a functional equivalent of the hypothalamic-pituitary-adrenal axis and synthesize cortisol. FASEB J. 2005 Aug;19(10):1332-4 Department of Dermatology, Hamamatsu University School of Medicine, Hamamatsu, Japan
CRH => ↑ Alpha, MSH, ACTH in hair

Figure 2. Immunoreactivity (IR) of key components of the HPA axis in organ-cultured human scalp hair follicles. After

Ito N, Ito T, Kromminga A, Bettermann A, Takigawa M, Kees F, Straub RH, Paus R. Human hair follicles display a functional equivalent of the hypothalamic-pituitary-adrenal axis and synthesize cortisol. FASEB J. 2005 Aug;19(10):1332-4 Department of Dermatology, Hamamatsu University School of Medicine, Hamamatsu, Japan
MSH => darkens coat color of adult mice

SUBJECTS: Agouti mice C3H Avy => coat color of dorsum changes from birth to maturity: young => dorsal tegument = yellow ⇔ adult mice: shifts to dark gray.

- RESULTS:
  - bromoergocryptine, a dopamine agonist, as a single inj. in a beeswax pellet => prevented the color change resulting in the persistence of the immature pattern
  - After plucking an area of the dorsum of an adult animal, => regrown hair was dark;
  - however, when bromoergocryptine at the time of plucking => the new hair was not dark but yellow.
  - MSH inj. in adult animals previously treated with the drug=>'bleaching' effect of bromoergocryptine was abolished
  - Bromoergocryptine-treated mice => decreased MSH content of the pars intermedia & signs of hypoactivity in cells of this lobe

CCL: drug effect on coat color => due to inhibition of the secretion of MSH

To further investigate the mechanism of hair pigmentation in the absence of POMC peptides, we studied wild-type (Pomc+/+), heterozygous (Pomc+/-), and homozygous (Pomc-/-) mice on a nonagouti (a/a) 129;B6 hybrid genetic background. All three genotypes had similar black fur pigmentation with yellow hairs behind the ears, around the nipples, and in the perianal area characteristic of inbred C57BL/6 mice. Histologic and electron paramagnetic resonance spectrometry examination demonstrated that hair follicles in back skin of Pomc-/- mice developed with normal structure and eumelanin pigmentation; corresponding molecular analyses, however, excluded local production of alpha MSH and ACTH because neither Pomc nor putative Pomc pseudogene mRNAs were detected in the skin. Thus, 129;B6 Pomc null mutant mice produce abundant eumelanin hair pigmentation despite their congenital absence of melanocortin ligands. These results suggest that either the mouse melanocortin receptor 1 has sufficient basal activity to trigger and sustain eumelanogenesis in vivo or that redundant nonmelanocortin pathway(s) compensate for the melanocortin deficiency. Whereas the latter implies feedback control of melanogenesis, it is also possible that the two mechanisms operate jointly in hair follicles.

Prolactin, not α-MSH => stimulates color of winter fur in hamsters

Chronic implants of testosterone in castrated males delayed and attenuated the short photoperiod-induced coat color change. Administration of ovine prolactin (100 micrograms/day) stimulated pigmentation in hamsters with the winter pelage, whereas administration of α MS (30 micrograms/day) was without effect. These results suggest that changes in pelage color may be regulated largely by changes in pituitary prolactin secretion and modified to some extent by changes in gonadal steroid hormone secretion.

MSH Deficiency?

early graying, ...
ACTH:

Treatment of Gray or White Hair?
Proopiomelanocortin

=> α-MSH, γ-MSH, ACTH, etc;

Figure Structure of the POMC gene showing the MSH transgene in gray.
40 young male volunteers with different pigmentation levels (Caucasians: skin types I, II, III. Blacks: skin type VI). Significantly lower mean basic values of alpha-MSH and ACTH of light-haired persons in comparison with dark-haired and black persons (p < 0.05) were demonstrated. Significant increase of these proteo-hormones (alpha-MSH: skin type I: 26.7%, skin type II: 22.7%) in persons less pigmented within a short time after UV A whole-body irradiation in contrast to the more pigmented volunteers. These results prove a cutaneous peripheral sensor for UV A-rays, reacting with a different sensitivity depending on disposition and inducing endocrinological reactions. How this cutaneous-hypothalamic-pituitary stimulus mediation functions in detail is not completely revealed up to now. In what respect the present results, which can be explained as a consequence of evolutionary development, have a connection with the induction of melanoma remains to be seen.

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Fig. 1.9 Des granules de kératoxyline et des kératosomes sont présents dans la couche granuleuse; la structure lamellaire des kératosomes est visible (l'agrandissement). ME, x17500; x48000 (l'agrandissement).

Fig. 1.10 Les mélanocytes apparaissent comme des cellules claires dans la couche basale de l'épiderme. À gauche, pigment abondant sur une peau d'un sujet noir. À droite, coloration du pigment mélanique sur d'un simple lentigo chez une jeune femme. Coloration par l'H&E (à gauche) par la réaction de Masson-Fontana (à droite).
MSH
ACTH
testosterone
GH
**UV radiation => ↑MC-1R & POMC in human epidermis**

**Figure:** Upregulation of MC-1R, POMC, & IL-10 mRNA after UV irradiation *in vivo.* The volar aspects of the forearm of five healthy volunteers were left unirradiated (control) or were irradiated with 2MED using a solar simulator. Suction blisters were induced 3 h or 6 h after irradiation, and total RNA from epidermal roofs was subjected to reverse transcriptase–PCR. (a) Amplification products of MC-1R, POMC, IL-10, and β-actin were separated on agarose gels, and (b) evaluated densitometrically to semiquantify mRNA expression. Results are expressed as β-actin normalized relative mRNA levels (mean±SD) of five different experiments. *p<0.05 versus control. The absence of superscript means the differences were not statistically significant.

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Pituitary gland, ACTH immunoperoxidase stain (brown), x132
Thyroid hormones

=>

Reduces graying
of the hair
Gray Hair

due to:

slowing or failure in the repopulation of the new hair bulb in anagen phase with “fresh” melanocytes derived from the outer root sheath of the hair

Loss of pigmentation in the hair shafts in gray hairs ≠ due to complete loss of all follicular melanocytes

Melanocytes of the hair follicles reach their peak proliferative capacity during the early & intermediate anagen phase, which is associated with expression of c-kit on the surface of the melanocytes.

Loss of pigmentation in the hair shafts in gray hairs ≠ due to complete loss of all follicular melanocytes;

In fact, melanocytes negative for dopa & many other markers have been found in the outer root sheath of senile white hairs. (Horikawa T, Norris DA, Johnson TW, Zekman T, Dunscomb N, Bennion SD, et al. DOPA-negative melanocytes in the outer root sheath of human hair follicles express premelanosomal antigens but not a melanosomal antigen or the melanosome-associated glycoproteins tyrosinase, TRP-1, and TRP-2. J Invest Dermatol. 1996;106: 28-35.)

= due to lack of thyroid hormones??

CCL: The door is open to the possibility of stimulating the migration & differentiation of melanocytes to favor repigmentation of gray hair follicles

Redondo P et al. Repigmentation of Gray Hair After Thyroid Hormone Treatment. Actas Dermosifiliogr. 2007;98:603-10
PATIENT 1: T4 repigments gray hair completely in a 63-yr old

PATIENT 1: HYPOTHYROIDISM treated with high doses of T4
=> 63-year-old man, => progressively graying over the years & who had a 1-year history of fungoid mycosis (stage IA) treated with psoralen-UV-A (PUVA) (3 sessions/wk => myxedema coma of unknown cause.

CLINICAL SIGNS: a low level of consciousness, irregular breathing, inflammation, reddening of the face and both hands, and periorbital edema. systolic BP = 110 mmHg; diastolic BP= 60 mm Hg; pulse rate, 56 beats/min; & body temperature, 35ºC.

LAB TESTS:
• free T3 = 0.4 pg/mL (normal range, 1.5-4.3 pg/mL),
• free thyroxine (T4) = 1.8 pmol/mL (normal range, 10.5-25.0 pmol/mL),
• TSH = 102 mU/L (normal range, 0.45-7.0 mU/L).

TREATMENT: intensive care unit (ICU) for 10 days, => IV therapy of 0.5 mg of L-thyroxine => continued oral therapy with 0.1 mg of L-thyroxine. => return home after a month.

RESULT: On discharge from the ICU, complete repigmentation of all gray hairs of his scalp was observed & retained their coloration during 2 years of follow-up. The patient did not receive any other medication while he was in hospital.

Redondo P et al. Repigmentation of Gray Hair After Thyroid Hormone Treatment Actas Dermosifiliogr. 2007;98:603-10
PATIENT 2: hyperthyroidism repigments gray hair completely in a 51-yr old

PATIENT 2: Hyperthyroidism

A 51-year-old man + Cowden syndrome + history of thyroid carcinoma treated by surgery & thyroid hormone replacement therapy. His hair had gone progressively gray over time => 2 years later, he developed non-Hodgkin lymphoma of the tonsils & cervical lymph nodes, which was treated with chemotherapy + radiotherapy (total dose, 36 Gy) => At the time, 60% of his hair was white

CLINICAL SIGNS: After 4 cycles of chemotherapy (cyclophosphamide, doxorubicin, vincristine, prednisone, and bleomycin) he presented in the clinic suffering from nervousness, anxiety, & insomnia. He had lost 15 kg. He was diagnosed with iatrogenic hyperthyroidism caused by hormone replacement therapy (0.2 mg/d of L-thyroxine).

LAB TESTS: free T3 levels of 1.62 pg/mL, free T4 levels of 34.4 pmol/L, & undetectable TSH (<0.1 mU/L) => During this time, complete repigmentation of his hair occurred

The levels of free T4 were normal 3 months after daily treatment with 0.15 mg of L-thyroxine. The pigmentation of his hair has persisted during the 3 years of follow-up.
PATIENTS 1 & 2: high dose of T4 & T3 repigments gray hair in men > 50 yrs

PATIENT 1& 2:
Both patients received high doses of thyroid hormone,
• one due to myxedema coma
• the other due to overcompensation during thyroid hormone replacement therapy.
• Although other causes for the reversal of the graying processes cannot be ruled out, such as for example progression of cutaneous lymphoma or PUVA therapy in the first patient,11 or chemotherapy in the second one,12 hair pigmentation due to thyroid hormone seems the most plausible and reasonable explanation.
• In the first patient, repigmentation cannot be attributed to psoralens or UV-A, as this treatment was being performed for months before any affect on hair color was noted.
T3 \( \Rightarrow \) stimulates mouse hair growth & reduces gray hair in vitro

Figure 1. 7-week-old female C57BL/6 mice that have been depilated with cold wax after application of surface anesthesia with ketamine at a normal dose. An even pink coloration of the bare skin can be seen, characteristic of a synchronous telogen phase.

Redondo P et al. Repigmentation of Gray Hair After Thyroid Hormone Treatment Actas Dermosifiliogr. 2007;98:603-10
T3 => stimulates hair growth & reduces gray hair in vivo in mice

Figure Masson trichromic staining of the biopsy performed at day 10.

(A) Control mouse: follicles in the anagen phase (IV) in which the tip of the new hair shaft still had not reached the epidermis (.100).

(B) Topical T3-treated group: a higher follicular density can be seen in a more advanced anagen phase (VI), with hair protruding through the epidermis (.100).

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T3 \( \rightarrow \) stimulates mouse hair growth & reduces gray hair in vitro

**Study**: Application was daily, and the study lasted 10 days.

After 5 days of treatment \( \rightarrow \) a change in the skin coloration of the mouse treated with topical T3 (right suggesting **synchronous entry into the anagen phase**).

**Formula**: 0.5 µg of T3 dissolved in 20 µL of ethanol was applied to the dorsum of all treated mice, received locally 20 µL of ethanol.

*Redondo P et al. Repigmentation of Gray Hair After Thyroid Hormone Treatment*  
*Actas Dermosifiliogr.* 2007;98:603-10
Link Hypothyroidism => Premature Graying

Thyroid hormones participate in the maturation of cells derived from the neural crest through regulation of $\beta_2$-adrenergic receptors. Melanocytes are derived from the neural crest. => Decreases in thyroid hormone levels affect melanocytes, as occurs with other cells derived from the neural crest.

At times, sudden hair whitening may be preceded by a stressful experience or neuropsychiatric disorders, often related with decreased thyroid hormone or increased demand. It is popularly accepted that premature graying over a short period of time is caused by worries and being in a state of tension. Thus, events such as a fright, a stressful episode, and sustained anxiety (situations in which the appearance of alopecia areata or sudden graying of hair is widely documented both historically and culturally) may lead to a sudden increase in demand for T3. In situations in which it is impossible to produce more hormone, the first cells affected may be the follicular melanocytes.

Epidermal melanogenesis is constant and may have lower hormonal demands ⇔ follicular melanogenesis is cyclic, momentary, & may have a higher hormonal demand.

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Thank you for your attention!