

# **BENEFITS OF ACTIVE THERMAL EXERCISE**

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## ABSTRACT: BENEFITS OF ATE

### (ACTIVE THERMAL EXERCISE)

**ABSTRACT:** “Active Thermal Exercise” (“ATE”) includes cardiovascular (aerobic), strength or flexibility exercise performed in high temperature environments. Scientific research has established that the synergistic effect of exercise done in a heated environment causes a number of physiological changes which result in multiple benefits including the following:

1. Heat Acclimatization/Acclimation
2. Cardiovascular Changes
3. Biochemical Changes
4. Benefits for the Brain
5. Benefits for the Muscles
6. Improved Body Composition
7. Greater Longevity
8. Heat Acclimatization/Acclimation

Both exercise and heat exposure provide most of the foregoing benefits, but in combination the physiological changes and improvements which help the body handle the resulting stress and increase in core temperature are significantly magnified. These physiological modifications include increased heart rate (during the activity—up to 150 b/p/m), stroke volume, plasma volume, red blood cell count, ventricular compliance, sweat rate (26% increase), blood flow and oxygen transport to skeletal muscle, skeletal muscle force generation, myocardial efficiency and neurogenesis. Additional physiological improvements include reduced oxygen uptake, muscle glycogen sparing, and reduced blood lactate, insulin blood sugar levels and glycogen depletion. ATE also causes increased production of both glucose and fat-burning enzymes, increased caloric consumption and protein synthesis as well as increased release of human growth hormone (HGH), prolactin, endorphins, brain-derived neurotrophic factor (BDNF), heat shock proteins (HSPs) and oxidative stress proteins. The process by which the human body makes physiological changes to reduce the stress of the environment is called “acclimatization”. Studies have shown that ATE results in improved responses to exercise in both hot and temperate conditions that are similar to the improvements observed with strenuous training regimens.

ATE long-term benefits therefore include increased muscle mass, reversal of age-related muscle atrophy, increased longevity, improved body composition, enhanced learning, pain relief and stress management. Muscle mitochondria has been shown to increase 2-3 fold. Heat also enables users to perform exercises more safely and effectively, and facilitates flexibility by warming up the muscles, ligaments and joints. Heat also allows greater fluidity and range of motion. In addition, the muscles and connective tissue become more elastic, allowing for improved flexibility with less chance of injury and improved resolution of injuries. Thermal exposure combined with exercise increases metabolic rate and caloric consumption during the workout—and after. ATE helps exercisers achieve higher energy levels and reduce body weight and body mass index (“BMI”). Additional benefits include better sleep, lower fat “set-points” (helping with the achievement of long-term weight reduction), and reduced heart rates, cholesterol, triglycerides and blood sugar levels.

## Human Physiology Laboratory Review: THE MANY BENEFITS OF ACTIVE THERMAL EXERCISE

- Heat acclimatization benefits
- Cardiovascular benefits
- Biochemical benefits
- Benefits for the brain
- Benefits for the skeletal muscles
- Longevity benefits

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# **Heat Acclimation Benefits from Hyperthermic Conditioning and Active Thermal Exercise**

## **Introduction**

Research has shown heat acclimation can cause substantial improvements in the physiology of body and brain, similar to the enhancement of aerobic performance which occurs from altitude acclimatization.

**Hyperthermic conditioning is passive exposure to high temperature and “Active Thermal Exercise” (“ATE”) includes cardiovascular (aerobic), strength or flexibility exercise performed in high temperature environments.**

Exercise in heat, as compared with an ambient-neutral environment, causes changes in the dynamics of the human body, including alterations in the the circulatory, thermoregulatory and endocrine systems.(32) A number of interrelated physiologic processes work in concert to keep the core temperature stable, maintain central blood pressure and muscular function , and regulate fluid volume.(32)

The numerous physiological gains from heat acclimation include reduced oxygen uptake, muscle glycogen sparing, reduced blood lactate levels, increased skeletal muscle force generation, plasma volume expansion, improved myocardial efficiency, and increased ventricular compliance.

Studies have established that the effect of controlled passive high heat exposure and/or exercise done in a controlled high heat environment stimulates a number of physiological changes which result in multiple benefits including the following:

1. Heat Acclimatization/Acclimation
2. Cardiovascular Changes
3. Biochemical Changes
4. Benefits for the Brain
5. Benefits for the Muscles
6. Improved Body Composition
7. Greater Longevity

## I. HEAT ACCLIMATIZATION/ACCLIMATION

Acclimatization (or acclimation) is the process by which the human body makes physiologic adaptations to reduce the stress of an environment. (31) Heat acclimatization refers to an organism's ability to survive an otherwise lethal heat stress from a prior heat exposure sufficient to cause the cellular accumulation of heat shock proteins.(24) Studies have shown that a period of 9 to 10 days is generally sufficient to attain most of the physiologic benefits associated with acclimatization,(31) and that physical endurance for exercise in hot, dry environments appears to be limited by the attainment of a critical level of core temperature.(5) High core temperature-- and not circulatory failure or metabolic depletion-- is the critical factor in heat stress, both before and after acclimation (5) Heat acclimatization results from a series of elevations in core temperature, generated by performing work in the heat, (24) and results in a number of physiological changes including the following:

A. Improved thermo-regulatory control: Thermoregulatory control is improved via activation of the sympathetic nervous system and the resulting increases in the flow of blood to the skin and the rate of sweating.(2) Acclimatization to work in the heat brings an earlier onset of sweating, increase in sweat rate and evaporative cooling that reduces heart rate in proportion to decreased core temperature.(2,45) After acclimation, sweating occurs at a lower core temperature and the sweat rate is maintained for a longer time period.(2)

B. Reduced resting core temperature and greater heat-dissipating capacity: Heat exposure causes a cascade of cardiovascular adaptations to heat including higher heart rates.(33) Heat acclimation reduces resting core temperature and increases heat-dissipating capacity. (24) Heat acclimation has also been shown to increase stroke volume, plasma volume (by 13%) and sweat rates.(5,29,32)

C. Greater ability to dissipate excessive body heat and maintain lower core temperature: ATE lengthens the time before the core temperature reaches 40 degrees C.(1) The resulting

improvements in evaporative cooling enhances the dissipation of metabolic heat during exercise in heat.(29)

D. Prolongs ability to continue exercising before exhaustion: Trained athletes generally reach the point of exhaustion when core temperatures reach 39 degrees Celsius.(34) Heat acclimatization allows the organism to tolerate a higher core temperature and therefore prolongs the ability to continue exercising before exhaustion. (24) A study using a climatic chamber to study exercise in dry heat found that acclimation increased average endurance before reaching exhaustion of the study subjects from 48 minutes to 80 minutes.(5)

E. Reduced lactate accumulation: Studies have also shown that heat acclimation results in reduced lactate accumulation in blood and muscle.(3)

F. Results in increased intracellular heat shock proteins (HSPs): HSPs are involved in maintaining cellular protein conformation and homeostasis during stress.(23, 24). The increase in HSPs resulting from heat acclimation is illustrative of a **cellular** adaptation to repeated heat stress in humans.(23)

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## II. CARDIOVASCULAR CHANGES

Exercise- and heat conditioning- cause the core temperature to increase. To cool the body, blood is sent to the skin to transfer the heat from the core to the skin. The process of perspiration causes evaporation from the skin to cool the blood before it is returned to the core.(29) This process is called “thermo-genesis” and results in increased heart rate, stroke volume and cardiac output at any given exercise intensity.(29) In sufficiently hot and/or humid environments, the process occurs even without exercise. If heat is not dissipated, the core temperature will increase and the subject will experience fatigue and exhaustion.(29) Exercise combined with heat exposure increases body temperature and activates beneficial physiological responses more significantly than either exercise or heat alone.(32)

Cardiovascular improvements which help to maintain a stable core temperature include the following:

A. Increased stroke volume: The amount of blood pumped by the left ventricle of the heart in a single contraction. Increased stroke volume reduces cardiovascular strain and lowers the heart rate for the same given workout.(2) Exercising in heat causes acclimation and increases stroke volume more than just exercise or heat exposure alone.(5,32)

B. Increased heart rate and cardiac output: ATE can increase heart rate up to 100 beats per minute with moderate heat exposure and/or exercise intensity and up to 150 beats per minute with high heat exposure and/or exercise intensity.(97) Cardiac output is also increased with ATE.(29) Exercising in a hot environment increases cardiac output more than just exercise or heat exposure alone.(32) Studies have shown that hyperthermic conditioning causes rises in cardiac output proportionally to increase in heart rate, and can increase cardiac output. by as much as **75%!** (108)

C. Increased sweating rate: The rate of sweating is increased with both exercise and heat exposure. A study with 12 fit subjects exercising to exhaustion at 95 degrees F. (and 87% relative humidity) showed a 26% increase in sweating rate.(91) Heat acclimation increases the size of the eccrine sweat glands -- and larger glands produce more sweat.(41) Thermal exposure combined with exercise results in greater increases in sweating than passive heat exposure alone.(32) Exercising in heat can cause sweat loss of from 2 to 6 pounds (1 to 3 liters) per hour, and each vaporized liter of sweat extracts 580 calories from the body! (29)

D. Increased sweat sensitivity: Sweat sensitivity determines the body's potential for evaporative cooling.(6) Sweat sensitivity increases during both heat acclimation and exercise conditioning.(32)

E. Increased core temperature: A rise in core temperature triggers the body's temperature regulating center for heat dissipation. Numerous studies have shown that exercise in hot, dry



environments is limited by the attainment of a critical level of core temperature and that high core temperature, not circulatory failure or metabolic depletion, is the critical factor in heat stress.(5, 121)

F. Increased blood flow to muscles: ATE increases the flow of blood to the skeletal muscles which keeps them fueled with glucose, fatty acids, and oxygen. At the same time, metabolic by-products such as lactic acid are more effectively removed. Improved delivery of nutrients reduces muscles dependence on glycogen stores, which helps endurance athletes perform for longer periods.

G. Increased blood plasma volume and red blood cell count (RBC): ATE has been shown to increase blood plasma volume by as much as 7.1% (13% in another study(5)) and increase red blood cell count (RBC) by 3.5%.(1) Increases in RBC results in increased the delivery of oxygen to the muscles.

H. ATE reduces muscle glycogen use: Studies have shown that ATE reduces muscle glycogen use by 40 to 50% before heat acclimation.(7,8) It is believe that reduced muscle glycogen use results from the increased flow of blood to the muscles.(7)

I. Enhanced endurance: The cardiovascular improvements described above have been shown to enhance endurance in both trained and untrained test subjects.(2,3,4) Heat acclimatization allows the organism to tolerate a higher core temperature and therefore prolongs the ability to continue exercising before exhaustion. (24) A study using a climatic chamber to study exercise in dry heat found that acclimation increased average endurance before reaching exhaustion of the study subjects from 48 minutes to 80 minutes.(5)

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### III.BIOCHEMICAL CHANGES

A. Reduced rate of glycogen depletion: When glycogen levels are low, muscles use protein and amino acids to produce glucose.(29) Protein and amino acids are the building blocks of muscle.(29) With shortages of glycogen, muscle starts using vital protein and amino acids for energy purposes.(29) This leads to muscle damage and overtraining (it has been shown that muscle damage limits and interferes with glycogen storage and synthesis). (29) Glycogen is the storage form of glucose + carbohydrates. About 80% of total carbohydrate is stored in skeletal muscle (about 14% is stored in the liver and 6% in the blood in the form of glucose).(29)

Glycogen is important but humans have a limited capacity to store it.(29) Muscle glycogen is crucial for ATP re-synthesis during exercise.(29) Studies show that exercising in hot environments reduces muscle glycogen use by 40 to 50% and show reduced rates of glycogen depletion due to improved muscle perfusion.(7,8). Additional studies show that heat acclimation leads to sparing of muscle glycogen associated with enhanced ability to perform highly intense exercise following prolonged exertion in the heat.(7)

B. Increased release of **human growth hormone\* (HGH)**: HGH is a vital hormone that affects the muscle loss and atrophy that typically occurs with aging.(12,13) The higher your levels of HGH, the healthier and stronger you will be. For most people, at about the age of 30 a stage called “somatopause” is reached. When this point is reached, HGH levels begin to drop off dramatically. This decline in HGH levels contributes to the aging process, so the maintenance of high HGH levels is increasingly important as we age.(43) A study has shown that exercise in a warm environment induced significant elevations in HGH concentrations (exercise induced elevations of plasma HGH levels with increments exceeding 20 ng/ml in 29 degree C. water and 30 ng/ml in 36 degree C. water).(68)

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\*Note: See appendix for additional information.

Studies have documented that hyperthermic conditioning can significantly induce the release of human growth hormone (HGH). (68,11,12,13) One study showed a **doubling** of HGH levels

with only two 20-minute heat sessions at 176 degrees F.(11,12) A second study showed that HGH levels can be **increased fivefold** with only two 15-minute heat-conditioning sessions,(11,12) and a third study showed that two one hour heat sessions each day at 176 degrees F. for one week increased HGH levels by **sixteen times** on the third day.(13) When hyperthermia and exercise are combined, the synergistic effect causes even greater increases in HGH.(93)

C. Increased protein synthesis: Stimulation of the uptake of amino acids into muscle cells increases protein synthesis. Exercise in heat has been shown to contribute to improved protein synthesis.(11,14,15)

D. Inhibited cellular protein degradation (and enzymes responsible for same): Hyperthermic conditioning and exercise in heat contribute to improved regulation of protein metabolism.(11,14,15,18)

E. Reduced blood lactate levels: Reduced lactate levels result from incomplete glucose burning because the cardiovascular system cannot furnish enough oxygen to break down pyruvic acid). Pyruvic acid is converted to lactic acid. (29) Increased levels of lactate in muscles causes fatigue during exercise. Reduced lactate production can increase the capacity for prolonged physical activity (it is believed that this is because of the increased blood flow to the muscles). (29) Exercise performed in a hot environment has been shown to reduce blood lactate levels.(16)

F. Increased concentrations of heat shock proteins (HSPs): HSPs and variations in the HSP70 gene can reduce protein degradation and promote muscle growth. HSPs also provide longevity and anti-aging benefits. (36,37,38,39) A growing body of literature supports the role of heat shock proteins in heat adaptation which allows organisms to perform work in high-temperature environments.(24)

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G. Increased prolactin release: Prolactin is a hormone produced in the pituitary gland. Named originally after its function to promote milk production (lactation) in mammals, it has since been shown to have more than 300 functions (reproductive, metabolic, fluid regulation, regulation of the immune system and behavior). Prolactin is an indirect marker of central fatigue.(49) A study comparing the prolactin responses of subjects reaching exhaustion via cycling to subjects heated to the same core temperature passively found that with both forms of heating the prolactin response was the same. The conclusion is that core temperature is the key stimulus for prolactin release.(20)

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H. Reduced insulin resistance. Hyperthermic conditioning has been shown to protect skeletal muscle from high-fat diet–induced insulin resistance and provide strong evidence that HSP induction in skeletal muscle could be a potential therapeutic treatment for obesity-induced insulin resistance.(50)

#### **IV.BENEFITS FOR THE BRAIN**

A. Increased levels of prolactin: Prolactin is important for the promotion of myelin growth (which helps the brain function faster and repair nerve cell damage).(49) Studies have compared the prolactin responses of subjects reaching exhaustion via cycling to subjects heated to the same core temperature passively. It was found that with both forms of heating the prolactin response was the same. The conclusion is that core temperature is the key stimulus for prolactin release.(20)

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B. Increased endorphin levels: Brain chemicals known as neurotransmitters include “endorphins”, which function to transmit electrical signals within the nervous system. At least 20 types of endorphins have been demonstrated in humans. Endorphins can be found in the pituitary gland and in other parts of the brain, or distributed throughout the nervous system.(122) Stress and discomfort (pain) are the two most common factors leading to the release of endorphins. Endorphins interact with the opiate receptors in the brain to reduce

perception of pain (and act similarly to drugs such as morphine and codeine). In addition to decreased feelings of pain, secretion of endorphins leads to feelings of euphoria, modulation of appetite, release of sex hormones, and enhancement of the immune response.(122) The so-called “runner’s high” can result from a boost in endorphin levels, and the sense of well-being associated with intensive endurance athletics. Thermal conditioning and ATE also boost endorphin levels.(49) The boost in endorphin levels associated with running is believed to be related to heat stress. Animal studies have found that heat stress from thermal exposure can significantly increase endorphin levels.(22)

C. Increased **heat shock protein\* (HSP)** production: When injury occurs to a part of the brain, such as stroke or traumatic injury, HSP production is often increased to repair damage (95)

D. Increased **brain-derived neurotrophic factor\* (BDNF)**: Research has established that exercise triggers the production of BDNF, which helps support the growth (and survival) of existing brain cells and the development of new ones (certain types of exercise have been shown to **triple** the synthesis of BDNF in the human brain)!(98). BDNF is a protein or “neuropeptide”- a member of the

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\* Note: See appendix for additional information.

neurotrophin family of growth factors—known to be important for long-term memory.(99) As humans age, BDNF levels typically fall. This decline is one of the main reasons brain function generally deteriorates in the elderly. Research has shown that exercise can help to counteract these age-related drops in BDNF and can restore young levels of BDNF in the aging brain. BDNF activates brain stem cells to produce new neurons and triggers other important chemicals. Increased neurogenesis is believed to enhance learning, long-term memory and cognitive function as well as ameliorate anxiety, depression, schizophrenia, epilepsy, Alzheimer’s disease, drug addiction, obesity and other conditions.(46) A recent study with 15 subjects showed increased levels of serum BDNF from baseline of 13% and 30% with cycle

ergometer exercise.(86) Another study with 11 subjects showed increased levels of serum BDNF which were enhanced with exercise in the heat. It was shown that heat stress increased the expression of BDNF more than exercise alone.(47) Since permeability of the blood–brain barrier increases with exercise in the heat, the opinion of the researchers was that thermal exercise causes a higher cerebral output of BDNF.(47)

E. Increases perfusion and size of hippocampus: The hippocampus generally shrinks in late adulthood, resulting in impaired memory and increased risk of dementia. A study with 120 older adults without dementia showed that exercise intervention increases cerebral blood volume and perfusion and the size of hippocampus.(87)

F. Improves cognitive processes and memory: Increased cerebral blood flow and oxygenation, in addition to increased levels of serum BDNF as shown above, can improve cognitive processes and memory.(65) Studies have shown that both hyperthermic conditioning (65) and exercise improve cognition and brain performance, including memory.(85, 86, 87)

G. Increases production of norepinephrine: Studies have shown that active thermal exercise and hyperthermic conditioning increase norepinephrine by as much as 310% (11,12) and 86% (48). Norepinephrine helps improve focus and attention to detail.(97)

## **V. BENEFITS FOR THE MUSCLES**

Our muscles are continually waging a war between the growth of new muscle cells (protein synthesis) and degradation of our existing proteins. The key factor is our **net protein synthesis** which takes into account both new protein synthesis and degradation. ATE reduces the amount of protein degradation taking place and therefore boosts net protein synthesis as follows:

A.ATE creates increased muscle mass:

1. Increased **heat shock proteins\* (HSPs)**: Heat acclimation increases net protein synthesis and muscle growth.(14,15) Increased production of heat shock proteins (HSPs) promotes muscle growth and reduces protein degradation.(14,15) Protein degradation occurs naturally

during both muscle use and disuse. HSPs induced by heat help to both prevent and repair damaged proteins. HSPs are used by the cells to counteract potentially harmful stimuli.(14,15) HSPs can prevent damage by scavenging free radicals and supporting cellular antioxidant capacities via their help in maintaining glutathione levels.(14,15) HSPs also repair misfolded and damaged proteins so proper structure and function is maintained.(14,15)

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\*Note: See appendix for additional information

2. Increased **muscle mitochondria**\*: Research has shown that both heat exposure and high intensity exercise cause heat shock and oxidative stress (generation of  $O_2^-$  and  $H_2O_2$ ). In addition, both exercise and ATE training promote mitochondrial biogenesis (2–3-fold increases in muscle mitochondria). (23,24,25).

3.Increased levels of **human growth hormone\* (HGH)**: ATE increases muscle growth by large induction of HGH.(12,13,5) Studies have shown that exercise in high heat (40 degrees C.) resulted in increased HGH concentrations from the resting value both in the first and last heat tests.(5) The studies also showed that resting aldosterone (HGH) concentration was increased after heat acclimation.(5) Another study showed that exercise in a heated (40 degrees C.) climatic chamber almost doubled plasma HGH from levels achieved with the same exercise done under thermo-neutral (23 degrees C.) conditions.(92) Studies have shown that the major anabolic effects of HGH in skeletal muscle may result from the inhibition of muscle protein degradation, which results in net increases in protein synthesis.(18) Another study concluded that the administration of HGH to athletes for four weeks decreased muscle protein oxidation and degradation by 50%.(28)

B. Increased production of muscle proteins: Exercise in heat contributes to improved protein synthesis.(11,14,15), and heat acclimation increases net protein synthesis and muscle growth.(14,15) Stimulation of the uptake of amino acids into muscle cells increases protein synthesis.(55) An animal study

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\*Note: See appendix for additional information.

utilizing intermittent hyperthermia induced significant HSP in skeletal muscle which augmented muscle growth by 30%.(14) The animal study also showed that increased HSP expression can persist for 48 hours after heat shock.(14,15)

C. Reduced protein degradation and protection against degenerative muscle tissue conditions: Muscle growth can be promoted by triggering the release of heat shock proteins (HSPs) which reduce the amount of protein degradation that naturally occurs during both muscle use and disuse.(14,15) Human growth hormone (HGH) also decreases protein degradation. Reduced protein degradation increases the net protein synthesis in the muscles and therefore promotes muscle growth.(14,15) It has also been shown that exercise in heat increases concentrations of HSPs, which may illustrate a cellular adaptation of heat acclimation in humans.(23) HSPs also help repair damaged proteins and help maintain proper protein structure and function, and thereby help protect against degenerative muscle tissue conditions.(14,15)

D. Reverses age-related muscle atrophy (sarcopenia): Sarcopenia [age-related loss of muscle] affects about 10 percent of those over 60, with higher rates as age advances. Causes of the loss of muscle mass or strength include hormonal changes, sedentary lifestyles, oxidative damage, infiltration of fat into muscles, inflammation and resistance to insulin.(49) Exercise in heat contributes to improved protein synthesis. (11,14,15) Exercise in heat increases concentrations of HSPs, which may illustrate a cellular adaptation of heat acclimation in humans. (23)

E. Reduces levels of lactic acid in the blood: Increased levels of lactate in muscles causes fatigue during exercise. Reduced lactate production can increase the capacity for prolonged physical activity. It is believed that this is because of increased blood flow to the muscles.(29) Exercise performed in a hot environment has been shown to reduce blood lactate levels.(16)

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F. Reduced muscle glycogen use: The reduced usage of glycogen by the muscles results from increased blood flow to the muscles.(7,8) Studies show that exercising in hot environments reduces muscle glycogen use by 40 to 50% and show reduced rates of glycogen depletion due to improved muscle perfusion. (7,8). Additional studies show that heat acclimation leads to sparing of muscle glycogen associated with enhanced ability to perform highly intense exercise following prolonged exertion in the heat.(7)

G. Increased lactate threshold: Increased levels of lactate in muscles causes fatigue during exercise. Reduced lactate production can increase the capacity for prolonged physical activity (it is believed this is because of increased blood flow to the muscles).(29) Exercise performed in a hot environment has been shown to reduce blood lactate levels.(16)

H. Improved recovery from muscle injury: To return to a healthy condition after injury, muscle regrowth must occur. Muscle regrowth after immobilization occurs as a result of elevated heat shock protein levels. **Brain-derived neurotrophic factor\*(BDNF)** is also secreted by muscle cells and plays an important role in muscle repair and growth.(30) Studies show that exercise increases serum

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\*Note: See appendix for additional information.

BDNF.(86) This increase can be enhanced with exercise in the heat. Since permeability of the blood–brain barrier increases with exercise in the heat, it is believed that this causes a higher cerebral output of BDNF.(47) Exercise in heat increases concentrations of HSPs, which may illustrate a cellular adaptation of heat acclimation in humans. (23)

I. Reduced neuro-motor degradation: Brain-derived neurotrophic factor (BDNF) also protects neuro-motors—the most critical elements in muscle-- from degradation.(60) Studies show that exercise increases serum BDNF. (86) This increase can be enhanced with exercise in the heat. Since permeability of the blood–brain barrier increases with exercise in the heat, it is believed that this causes a higher cerebral output of BDNF.(47)

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J. Improved insulin sensitivity: Insulin is an endocrine hormone responsible for promoting the uptake of glucose into muscle and adipose tissue. Insulin is also important for protein metabolism and increasing protein synthesis by stimulating the uptake of amino acids into the muscle.(94). In overweight individuals, insulin levels are elevated because the tissues do not respond properly to insulin (“insulin insensitivity”). This condition impedes the ability of glucose to enter muscle cells, causes high blood sugar levels and increases in the amount of glucose entering fat cells.(10,29) Studies have shown that ATE helps to reduce insulin resistance by improving insulin sensitivity and decreasing muscle protein catabolism. Animal studies have found that 30 minutes heat exposure three times per week for a period of 12 weeks can result in a 31 percent decrease in insulin levels.(10) Lower insulin levels help maintain higher sensitivity to insulin and promote the entry of glucose into muscle cells.(10,29) Exercise has been shown to significantly reduce the risk of developing insulin resistance by improving glucose tolerance and insulin action in individuals predisposed to develop type 2 diabetes.(50)

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## VI. IMPROVED BODY COMPOSITION

A.Exercise has been shown in numerous studies to improve body composition through reduced adiposity and improved weight control.(75-78, 88) Increased lean mass causes increased calorie burning.(29) Muscles burn over 90% of the Calories humans consume.(29)

B.Muscle has special enzymes that enable burning of large amounts of calories in short periods.(53)

C.Both exercise and heat exposure cause heat shock and oxidative stress (generation of  $O_2^-$  and  $H_2O_2$ ).

D.Both exercise and ATE training promote mitochondrial biogenesis (2–3-fold increases in muscle mitochondria) which leads to increased lean body mass. (23,24,25)

E.Hyperthermic conditioning has been shown to **triple** the synthesis of BDNF in the human brain. (98) Studies have also shown that BDNF is important for thermogenesis (the ability of cells to burn fat to produce heat) and for controlling appetite and satiety.(110)

## VII. GREATER LONGEVITY

A. ATE and greater longevity: A recent study published in *JAMA Internal Medicine* showed that thermal treatments are associated with greater longevity. The study of over 2,000 middle-aged men in Finland found that fatal cardiovascular disease was **27% lower** for men who used the sauna 2 to 3 times per week and **63% lower** in men taking 4 to 7 sauna sessions each week!(63)

B. Increased HSPs: HSPs and variations in the HSP70 gene can also help provide longevity and anti-aging benefits. In flies and worms, heat exposure has been shown to increase lifespans by as much as 15% (36,37,37.5, 38,39) A growing body of literature supports the role of HSPs in heat adaptation which allows organisms to perform work in high-temperature environments.(24) Other animal studies have shown that chronic exercise enhances HSP70 accumulation in skeletal muscle.(61) Exercise in heat has also been shown to increase concentrations of HSPs, which may illustrate a cellular adaptation of heat acclimation in humans. (23)

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C. **Foxo3 Gene\***: Another molecular pathway that may explain how heat exposure can improve longevity is a gene that is associated with longevity known as Foxo3\*. Foxo3 is one of the four mammalian Foxo genes, and it is activated by heat stress. Humans with a polymorphism that makes more of this gene have up to a 2.7fold increased chance of living to the age of 100.(97) In mice, having more of their homologous version of this gene can extend their lifespan by up to 30%.(96) The mechanism by which Foxo3 increases longevity has to do with the fact that it is a master regulator of many different genes. When the Foxo3 gene is “on”, it increases the expression of a number of genes that increase resistance to many of stressors that occur with aging. Many of the genes that foxo3 increases typically **decrease** with age, so it is important for longevity to boost their expression.(97) One critically important stress that Foxo3 protects against is DNA damage. The same type of reactive byproducts (from normal metabolism and immune function) that damage proteins in the cell also damage DNA.(97) DNA damage often

leads to mutations. Damaged cells with mutations often replicate to form cancer. Foxo3 increases the expression of DNA repair genes that help prevent cell mutations.(97) Foxo3 also increases the expression of genes that kill cell damaged cells so that they do not become cancer cells.(97) Foxo3 makes cells more resilient to damage by increasing the expression of genes that combat damage such as antioxidant genes which prevent the damage from reaching the cell. Finally, Foxo3 increases the expression of genes responsible for immune function (which generally declines with age). Boosting the immune system enables us to combat bacteria, viruses, and cancer cells more effectively which leads to longer and healthier lives.(97)

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\*Note: See appendix for additional information.

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## Brain-derived Neurotrophic Factor (BDNF)

**Brain-derived neurotrophic factor**, also known as **BDNF**, is a [protein](#)<sup>[2]</sup> that, in humans, is encoded by the *BDNF* [gene](#). BDNF is a member of the [neurotrophin](#) family of growth factors, which are related to the canonical [Nerve Growth Factor](#). [Neurotrophic factors](#) are found in the [brain](#) and the periphery. In the mid-1990's, research first showed that exercise triggers the production of BDNF, which helps support the growth of existing brain cells and the development of new ones. As humans age, BDNF levels typically fall. This decline is one of the main reason brain function generally deteriorates in the elderly. Research has shown that aerobic exercise can help to counteract these age-related drops in BDNF and can restore young levels of BDNF in the aging brain.

### Function

BDNF acts on certain [neurons](#) of the [central nervous system](#) and the [peripheral nervous system](#), helping to support the survival of existing neurons, and encourage the growth and [differentiation](#) of new neurons and [synapses](#).<sup>[5][6]</sup> In the brain, it is active in the [hippocampus](#), [cortex](#), and [basal forebrain](#)—areas vital to [learning](#), [memory](#), and higher thinking.<sup>[7]</sup> It is also expressed in the retina, motor neurons, the kidneys, saliva, and the prostate.<sup>[8]</sup>

BDNF itself is important for [long-term memory](#).<sup>[9]</sup> Although the vast majority of neurons in the [mammalian](#) brain are formed prenatally, parts of the adult brain retain the ability to grow new neurons from neural [stem cells](#) in a process known as [neurogenesis](#). Neurotrophins are chemicals that help to stimulate and control neurogenesis, BDNF being one of the most active.<sup>[10][11][12]</sup> Mice born without the ability to make BDNF suffer developmental defects in the brain and [sensory nervous system](#), and usually die soon after birth, suggesting that BDNF plays an important role in normal [neural development](#).<sup>[13]</sup> Other important neurotrophins structurally related to BDNF include [NT-3](#), [NT-4](#), and [NGF](#).

BDNF is generally produced in the [endoplasmic reticulum](#) and secreted from dense-core vesicles. It binds [carboxypeptidase E](#) (CPE), and the disruption of this binding has been proposed to cause the loss of sorting of BDNF into dense-core vesicles. The [phenotype](#) for BDNF knockout mice can be severe, including postnatal lethality. Other traits include sensory neuron losses that affect coordination, balance, hearing, taste, and breathing. Knockout mice also exhibit cerebellar abnormalities and an increase in the number of sympathetic neurons.<sup>[14]</sup>

[Certain types of physical exercise](#) have been shown to markedly (threefold) increase BDNF synthesis in the human brain, a phenomenon which is partly responsible for exercise-induced neurogenesis and improvements in cognitive function. Niacin appears to upregulate BDNF and [tropomyosin receptor kinase B](#) (TrkB) expression as well.<sup>[19]</sup>

### Mechanism of action

BDNF binds at least two receptors on the surface of cells that are capable of responding to this growth factor, [TrkB](#) (pronounced "Track B") and the [LNGFR](#) (for *low-affinity nerve growth factor receptor*, also known as p75).<sup>[20]</sup> It may also modulate the activity of

various neurotransmitter receptors, including the [Alpha-7 nicotinic receptor](#).<sup>[21]</sup> BDNF has also been shown to interact with the [reelin](#) signaling chain.<sup>[22]</sup> The expression of reelin by [Cajal-Retzius cells](#) goes down during development under the influence of BDNF.<sup>[23]</sup> The latter also decreases reelin expression in neuronal culture.

## TrkB

The TrkB receptor is encoded by the [NTRK2](#) gene and is member of a receptor family of tyrosine kinases that includes [TrkA](#) and [TrkC](#). These receptors all interact with neurotrophins in a [ligand](#)-specific manner. TrkB [autophosphorylation](#) is dependent upon its ligand-specific association with BDNF, a widely expressed activity-dependent neurotrophic factor that regulates [neuroplasticity](#) and is upregulated following [hypoxic](#) injury.

## LNGFR

The role of the other BDNF receptor, [p75](#), is less clear. While the TrkB receptor interacts with BDNF in a ligand-specific manner, all neurotrophins can interact with the p75 receptor.<sup>[24]</sup> When the p75 receptor is activated, it leads to activation of [NFkB](#) receptor.<sup>[24]</sup> Thus, neurotrophic signaling may trigger [apoptosis](#) rather than survival pathways in cells expressing the p75 receptor in the absence of Trk receptors. Recent studies have revealed a truncated isoform of the TrkB receptor (t-TrkB) may act as a dominant negative to the p75 neurotrophin receptor, inhibiting the activity of p75, and preventing BDNF-mediated cell death.<sup>[25]</sup>

## Expression

The BDNF protein is encoded by a gene that is also called BDNF, found in humans on chromosome 11.<sup>[3][4]</sup> Structurally, BDNF transcription is controlled by 8 different promoters, each leading to different transcripts containing one of the 8 untranslated 5' promoter exons spliced to the 3' encoding [exon](#). Promoter IV activity is strongly stimulated by calcium and is primarily under the control of a [Cre](#) regulatory component, suggesting a putative role for the transcription factor [CREB](#) and the source of BDNF's activity-dependent effects.<sup>[26]</sup> There are multiple mechanisms through neuronal activity can increase BDNF exon IV specific expression.<sup>[26]</sup> Stimulus-mediated neuronal excitation can lead to NMDA receptor activation, triggering a calcium influx. Through a protein signaling cascade requiring [Erk](#), [CaM KII/IV](#), [PI3K](#), and [PLC](#), [NMDA](#) receptor activation is capable of triggering BDNF exon IV transcription. BDNF exon IV expression also seems capable of further stimulating its own expression through TrkB activation. BDNF is released from the post-synaptic membrane in an activity-dependent manner, allowing it to act on local TrkB receptors and mediate effects that can leading to signaling cascades also involving Erk and CaM KII/IV.<sup>[26][27]</sup> Both of these pathways probably involve calcium-mediated phosphorylation of CREB at Ser133, thus allowing it to interact with BDNF's Cre regulatory domain and upregulate transcription.<sup>[28]</sup> However, NMDA-mediated receptor signaling is probably necessary to trigger the upregulation of BDNF exon IV expression because normally CREB interaction with CRE and the subsequent translation of the BDNF transcript is blocked by of the [basic helix-loop-helix](#) transcription factor protein 2 ([BHLHB2](#)).<sup>[29]</sup> NMDA receptor activation triggers the release of the regulatory inhibitor, allowing for BDNF exon IV upregulation to take place



in response to the activity-initiated calcium influx.<sup>[29]</sup> Activation of [Dopamine receptor D5](#) also promotes expression of BDNF in [prefrontal cortex](#) neurons.<sup>[30]</sup>

[Val66Met](#) (rs6265) is a [single nucleotide polymorphism](#) in the gene where [adenine](#) and [guanine alleles](#) vary, resulting in a variation between [valine](#) and [methionine](#) at [codon 66](#).<sup>[31][32]</sup> As of 2008, Val66Met is probably the most investigated [SNP](#) of the *BDNF* gene, but, besides this variant, other SNPs in the gene are C270T, rs7103411, rs2030324, rs2203877, rs2049045 and rs7124442.

## **BDNF also produced by skeletal muscle**

Research has also shown that skeletal muscle can produce BDNF in response to exercise. It was found that BDNF mRNA and protein expression was increased in human skeletal muscle after exercise; however; muscle-derived BDNF appeared not to be released into the circulation. In addition, BDNF mRNA and protein expression was increased in muscle cells that were electrically stimulated. Interestingly, BDNF increased phosphorylation of AMPK and Acetyl Cocarboxylase (ACC) and enhanced fat oxidation both in vitro and ex vivo. Thus, BDNF has been identified as a novel contraction -induced muscle cell-derived protein that may increase fat oxidation in skeletal muscle in an AMPK dependent fashion. BDNF appears to be a myokine that works in an autocrine or paracrine fashion with strong effects on peripheral metabolism, including fat oxidation with a subsequent effect on the size of adipose tissue.

## **Role in synaptic transmission**

### **Glutamatergic signaling**

[Glutamate](#) is the brain's major excitatory [neurotransmitter](#) and its release can trigger the [depolarization](#) of [postsynaptic](#) neurons. [AMPA](#) and [NMDA](#) receptors are two major [ionotropic receptors](#) that are especially suspected of being involved in learning and memory. While AMPA receptor activation leads to depolarization via sodium influx, NMDA receptor activation leads to depolarization via calcium and sodium influx. The calcium influx triggered through NMDA receptors can lead to the activity-dependent expression of many different genes, proteins, and receptors that are thought to be involved in processes involving learning, memory, neurogenesis, and environmental responses. The activity-dependent synaptic responses also lead to rapid insertion of AMPA receptors into the postsynaptic membrane, which will act to maintain ongoing glutamatergic transmission as sustained calcium influx could result in excitotoxicity

### **NMDA receptor activity**

NMDA receptor activation is essential to producing the activity-dependent molecular changes involved in the formation of new memories. Following exposure to an enriched environment, BDNF and NR1 phosphorylation levels are upregulated simultaneously, probably because BDNF is capable of phosphorylating NR1 subunits, in addition to its many other effects.<sup>[33][34]</sup> One of the primary ways BDNF can modulate NMDA receptor activity is through phosphorylation and activation of the NMDA receptor one subunit, particularly at the PKC Ser-897 site.<sup>[33]</sup> The mechanism underlying this activity is dependent upon both [ERK](#) and [PKC](#) signaling pathways, each acting individually, and



all NR1 phosphorylation activity is lost if the TrkB receptor is blocked.<sup>[33]</sup> PI3 kinase and Akt are also essential in BDNF-induced potentiation of NMDA receptor function and inhibition of either molecule completely eliminated receptor activity.<sup>[34]</sup> BDNF can also increase NMDA receptor activity through phosphorylation of the [NR2B](#) subunit. BDNF signaling leads to the autophosphorylation of the intracellular domain of the TrkB receptor (ICD-TrkB). Upon autophosphorylation, [Fyn](#) associates with the pICD-TrkB through its [Src homology domain 2](#) (SH2) and is phosphorylated at its Y416 site.<sup>[35][36]</sup> Once activated, Fyn can bind to NR2B through its SH2 domain and mediate phosphorylation of its Tyr-1472 site.<sup>[37]</sup> Similar studies have suggested Fyn is also capable of activating NR2A although this was not found in the hippocampus.<sup>[38][39]</sup> Thus, BDNF can increase NMDA receptor activity through Fyn activation. This has been shown to be important for processes such as spatial memory in the hippocampus, demonstrating the therapeutic and functional relevance of BDNF-mediated NMDA receptor activation.<sup>[38]</sup>

### **Synapse stability**

In addition to mediating transient effects on NMDAR activation to promote memory-related molecular changes, BDNF should also initiate more stable effects that could be maintained in its absence and not depend on its expression for long term synaptic support.<sup>[40]</sup> It was previously mentioned that [AMPA](#) receptor expression is essential to learning and memory formation, as these are the components of the synapse that will communicate regularly and maintain the synapse structure and function long after the initial activation of NMDA channels. BDNF is capable of increasing the mRNA expression of GluR1 and GluR2 through its interaction with the TrkB receptor and promoting the synaptic localization of [GluR1](#) via PKC- and CaMKII-mediated Ser-831 phosphorylation.<sup>[41]</sup> It also appears that BDNF is able to influence [Glu](#) activity through its effects on NMDA receptor activity.<sup>[42]</sup> BDNF significantly enhanced the activation of GluR1 through phosphorylation of tyrosine830, an effect that was abolished in either the presence of a specific [NR2B](#) antagonist or a trk receptor tyrosine kinase inhibitor.<sup>[42]</sup> Thus, it appears BDNF can upregulate the expression and synaptic localization of AMPA receptors, as well as enhance their activity through its postsynaptic interactions with the NR2B subunit. This suggests BDNF is not only capable of initiating synapse formation through its effects on NMDA receptor activity, but it can also support the regular every-day signaling necessary for stable memory function.

### **GABAergic signaling**

One mechanism through which BDNF appears to maintain elevated levels of neuronal excitation is through preventing [GABAergic](#) signaling activities.<sup>[43]</sup> While glutamate is the brain's major excitatory neurotransmitter and phosphorylation normally activates receptors, [GABA](#) is the brain's primary inhibitory neurotransmitter and phosphorylation of [GABAA receptors](#) tend to reduce their activity. Blockading BDNF signaling with a tyrosine kinase inhibitor or a PKC inhibitor in wild type mice produced significant reductions in spontaneous [action potential](#) frequencies that were mediated by an increase in the amplitude of GABAergic [inhibitory postsynaptic currents](#) (IPSC).<sup>[43]</sup> Similar effects could be obtained in BDNF knockout mice, but these effects were reversed by local application of BDNF.<sup>[43]</sup> This suggests BDNF increases excitatory synaptic signaling partly through the post-synaptic suppression of GABAergic of

signaling by activating PKC through its association with TrkB.<sup>[43]</sup> Once activated, PKC can reduce the amplitude of IPSCs through to GABAA receptor phosphorylation and inhibition.<sup>[43]</sup> In support of this putative mechanism, activation of PKC $\epsilon$  leads to phosphorylation of N-ethylmaleimide-sensitive factor (NSF) at serine 460 and threonine 461, increasing its ATPase activity which downregulates GABAA receptor surface expression and subsequently attenuates inhibitory currents.<sup>[44]</sup>

## Synaptogenesis

BDNF also enhances synaptogenesis. [Synaptogenesis](#) is dependent upon the assembly of new synapses and the disassembly of old synapses by  [\$\beta\$ -adducin](#).<sup>[45]</sup> Adducins are membrane-skeletal proteins that cap the growing ends of [actin](#) filaments and promote their association with spectrin, another cytoskeletal protein, to create stable and integrated cytoskeletal networks.<sup>[46]</sup> Actins have a variety of roles in synaptic functioning. In pre-synaptic neurons, actins are involved in synaptic vesicle recruitment and vesicle recovery following neurotransmitter release.<sup>[47]</sup> In post-synaptic neurons they can influence dendritic spine formation and retraction as well as AMPA receptor insertion and removal.<sup>[47]</sup> At their C-terminus, adducins possess a myristoylated alanine-rich C kinase substrate (MARCKS) domain which regulates their capping activity.<sup>[46]</sup> BDNF can reduce capping activities by upregulating PKC, which can bind to the adducing MARCKS domain, inhibit capping activity, and promote synaptogenesis through dendritic spine growth and disassembly and other activities.<sup>[45][47]</sup>

## Dendritogenesis

Local interaction of BDNF with the TrkB receptor on a single dendritic segment is able to stimulate an increase in PSD-95 trafficking to other separate dendrites as well as to the synapses of locally stimulated neurons.<sup>[48]</sup> [PSD-95](#) localizes the actin-remodeling GTPases, [Rac](#) and [Rho](#), to synapses through the binding of its PDZ domain to [kalirin](#), increasing the number and size of spines.<sup>[49]</sup> Thus, BDNF-induced trafficking of [PSD-95](#) to dendrites stimulates actin remodeling and causes dendritic growth in response to BDNF.

## Neurogenesis

BDNF plays a significant role in neurogenesis. BDNF can promote protective pathways and inhibit damaging pathways in the NSCs and NPCs that contribute to the brain's neurogenic response by enhancing cell survival. This becomes especially evident following suppression of TrkB activity.<sup>[24]</sup> TrkB inhibition results in a 2–3 fold increase in cortical precursors displaying EGFP-positive condensed apoptotic nuclei and a 2–4 fold increase in cortical precursors that stained immunopositive for cleaved [caspase-3](#).<sup>[24]</sup> BDNF can also promote NSC and NPC proliferation through [Akt](#) activation and [PTEN](#) inactivation.<sup>[50]</sup> There have been many in vivo studies demonstrating BDNF is a strong promoter of neuronal differentiation.<sup>[24][51]</sup> Infusion of BDNF into the lateral ventricles doubled the population of newborn neurons in the adult rat [olfactory bulb](#) and viral overexpression of BDNF can similarly enhance SVZ neurogenesis.<sup>[10][11][51]</sup> BDNF might also play a role in NSC/NPC migration. By stabilizing [p35 \(CDK5R1\)](#), in utero electroporation studies revealed BDNF was able to promote cortical radial migration by

about 2.3-fold in embryonic rats, an effect which was dependent on the activity of the trkB receptor.<sup>[52]</sup>

## Cognitive function

Enriched housing provides the opportunity for exercise and exposure to multimodal stimuli. The increased visual, physical, and cognitive stimulation all translates into more neuronal activity and synaptic communication, which can produce structural or molecular activity-dependent alterations.<sup>[53][54]</sup> Sensory inputs from environmental stimuli are initially processed by the cortex before being transmitted to the hippocampus along an afferent pathway, suggesting the activity-mediated effects of enrichment can be far-reaching within the brain.<sup>[54]</sup> BDNF expression is significantly enhanced by environmental enrichment and appears to be the primary source of environmental enrichments ability to enhance cognitive processes. Environmental enrichment enhances synaptogenesis, dendritogenesis, and neurogenesis, leading to improved performance on various learning and memory tasks. BDNF mediates more pathways involved in these enrichment-induced processes than any other molecule and is strongly regulated by calcium activity making it incredibly sensitive to neuronal activity.

## Disease linkage

See also: [Neuroplasticity](#) and [Neurobiological effects of physical exercise § BDNF signaling](#)

Various studies have shown possible links between BDNF and conditions, such as [depression](#),<sup>[55][56]</sup> [schizophrenia](#),<sup>[57]</sup> [obsessive-compulsive disorder](#),<sup>[58]</sup> [Alzheimer's disease](#),<sup>[59]</sup> [Huntington's disease](#),<sup>[60]</sup> [Rett syndrome](#),<sup>[61]</sup> and [dementia](#),<sup>[62]</sup> as well as [anorexia nervosa](#)<sup>[63]</sup> and [bulimia nervosa](#).<sup>[64]</sup> Increased levels of BDNF can induce a change to an [opiate-dependent-like](#) reward state when expressed in the [ventral tegmental area](#) in rats.<sup>[65]</sup>

## Schizophrenia

A plethora of recent evidence suggests the linkage between [schizophrenia](#) and BDNF.<sup>[66]</sup> Given that BDNF is critical for the survival of central nervous system (CNS) and peripheral nervous system (PNS) neurons and [synaptogenesis](#) during and even after development, BDNF alterations may play a role in the pathogenesis of [schizophrenia](#). BDNF has been found within many areas of the brain and plays an important role in supporting the formation of memories.<sup>[67]</sup> It has been shown that BDNF mRNA levels are decreased in cortical layers IV and V of the dorsolateral prefrontal cortex of schizophrenic patients, an area that is known to be involved with working memory.<sup>[68]</sup> Since schizophrenic patients often suffer from impairments in working memory, and BDNF mRNA levels have been shown to be decreased in the DLPFC of schizophrenic patients, it is highly likely that BDNF plays some role in the etiology of this neurodevelopmental disorder of the CNS.

## Depression

Exposure to [stress](#) and the stress hormone [corticosterone](#) has been shown to decrease the [expression](#) of BDNF in rats, and, if exposure is persistent, this leads to an eventual atrophy of the [hippocampus](#). Atrophy of the hippocampus and other limbic structures has been shown to take place in humans suffering from chronic [depression](#).<sup>[69]</sup> In addition, rats bred to be heterozygous for BDNF, therefore reducing its expression, have been observed to exhibit similar hippocampal atrophy. This suggests that an etiological link between the development of depression and BDNF exists. Supporting this, the excitatory neurotransmitter [glutamate](#), voluntary [exercise](#),<sup>[70]</sup> [caloric restriction](#), intellectual stimulation, [curcumin](#)<sup>[71]</sup> and various treatments for depression (such as [antidepressants](#)<sup>[72]</sup> and [electroconvulsive therapy](#)<sup>[73]</sup>) increase expression of BDNF in the brain. In the case of some treatments such as drugs<sup>[74]</sup> and electroconvulsive therapy<sup>[75]</sup> this has been shown to protect or reverse this atrophy.<sup>[74]</sup>

## Eczema

High levels of BDNF and [Substance P](#) have been associated with increased itching in [eczema](#).<sup>[76]</sup>

## Epilepsy

[Epilepsy](#) has also been linked with polymorphisms in BDNF. Given BDNF's vital role in the development of the landscape of the brain, there is quite a lot of room for influence on the development of neuropathologies from BDNF. Levels of both BDNF mRNA and BDNF protein are known to be up-regulated in epilepsy.<sup>[77]</sup> BDNF modulates excitatory and inhibitory synaptic transmission by inhibiting GABAA-receptor-mediated post-synaptic currents.<sup>[78]</sup> This provides a potential mechanism for the observed up-regulation.

## Alzheimer's disease

Post mortem analysis has shown lowered levels of BDNF in the brain tissues of people with [Alzheimer's disease](#), although the nature of the connection remains unclear. Studies suggest that neurotrophic factors have a protective role against [amyloid beta](#) toxicity.<sup>[79]</sup>

## Drug addiction and dependence

BDNF is a regulator of drug [addiction](#) and [psychological dependence](#). Animals chronically exposed to drugs of abuse show increased levels of BDNF in the ventral tegmental area (VTA) of the brain, and when BDNF is injected directly into the VTA of rats, the animals act as if they are addicted to and psychologically dependent upon opiates.<sup>[80]</sup>

## Obesity

In 2009, variants close to the BDNF gene were found to be associated with [obesity](#) in two very large genome-wide association studies of [body mass index](#) (BMI).<sup>[81][82]</sup>

## Aging

BDNF levels decrease during aging.<sup>[83]</sup>

## Congenital central hypoventilation syndrome

The polymorphism Thr2Ile may be linked to [congenital central hypoventilation syndrome](#).<sup>[84][85]</sup>

## Post-chemotherapy cognitive impairment

BDNF and IL-6 might be involved in the pathogenesis of [post-chemotherapy cognitive impairment](#) (PCCI, also known as chemo brain) and fatigue.<sup>[8]</sup>

# Human Growth Hormone (HGH)

**Growth hormone (GH or HGH)**, also known as **somatotropin** or **somatropin**, is a [peptide hormone](#) that stimulates [growth](#), [cell](#) reproduction and regeneration in humans and other animals. It is a type of [mitogen](#) which is specific only to certain kinds of cells. Growth hormone is a 191-[amino acid](#), single-chain [polypeptide](#) that is synthesized, stored, and secreted by [somatotrophic cells](#) within the lateral wings of the [anterior pituitary](#) gland.

GH is a [stress hormone](#) that raises the concentration of glucose and free fatty acids. It also stimulates production of [IGF-1](#). GH is used as a [prescription drug](#) in medicine to treat children's growth disorders and adult growth hormone deficiency. In the United States, it is only available legally from pharmacies, by prescription from a doctor. In recent years in the United States, some doctors have started to prescribe growth hormone in GH-deficient older patients (but not on healthy people) to increase vitality. While legal, the efficacy and safety of this use for HGH has not been tested in a clinical trial. At this time, HGH is still considered a very complex hormone, and many of its functions are still unknown.<sup>[3]</sup>

In its role as an [anabolic](#) agent, HGH has been abused by competitors in sports at least since 1982, and it has been banned by the [IOC](#) and [NCAA](#). Traditional [urine](#) analysis could not detect [doping](#) with HGH, so the ban was unenforceable until the early 2000s when [blood tests](#) that could distinguish between natural and artificial HGH were starting to be developed. Blood tests conducted by [WADA](#) at the [2004 Olympic Games](#) in [Athens, Greece](#) targeted primarily HGH.<sup>[3]</sup> This use for the drug is not approved by the FDA.

GH has been studied for use in raising livestock more efficiently in [industrial agriculture](#) and several efforts have been made to obtain governmental approval to use GH in livestock production. These uses have been controversial. In the United States, the only FDA-approved use of GH for livestock is the use of a cow-specific form of GH called [bovine somatotropin](#) for increasing milk production in dairy cows. Retailers are permitted to label containers of milk as produced with or without bovine somatotropin.

## Nomenclature

Somatotropin (STH) refers to the [growth hormone](#) produced naturally in animals and extracted from carcasses. Hormone extracted from human cadavers is abbreviated hGH. The growth hormone produced by [recombinant DNA technology](#) has the approved generic name somatropin and the brand name Humatrope,<sup>[4]</sup> and is properly abbreviated rhGH in the scientific literature. Since its introduction in 1992 Humatrope has been a banned sports doping agent,<sup>[5]</sup> and in this context is referred to as HGH.

## Biology

### Gene

Main articles: [Growth hormone 1](#) and [Growth hormone 2](#)



Genes for human growth hormone, known as [growth hormone 1](#) (somatotropin) and [growth hormone 2](#), are localized in the q22-24 region of chromosome 17<sup>[6][7]</sup> and are closely related to [human chorionic somatomammotropin](#) (also known as [placental lactogen](#)) genes. GH, human chorionic somatomammotropin, and [prolactin](#) belong to [a group of homologous hormones](#) with growth-promoting and lactogenic activity.

## Structure

The major isoform of the human growth hormone is a protein of 191 [amino acids](#) and a molecular weight of 22,124 [daltons](#). The structure includes four helices necessary for functional interaction with the GH receptor. It appears that, in structure, GH is evolutionarily homologous to prolactin and chorionic somatomammotropin. Despite marked structural similarities between growth hormone from different [species](#), only human and [Old World monkey](#) growth hormones have significant effects on the human [growth hormone receptor](#).<sup>[8]</sup>

Several [molecular](#) isoforms of GH exist in the pituitary gland and are released to blood. In particular, a variant of approximately 20 kDa originated by an alternative splicing is present in a rather constant 1:9 ratio,<sup>[9]</sup> while recently an additional variant of ~ 23-24 kDa has also been reported in post-exercise states at higher proportions.<sup>[10]</sup> This variant has not been identified, but it has been suggested to coincide with a 22 kDa glycosylated variant of 23 kDa identified in the pituitary gland.<sup>[11]</sup> Furthermore, these variants circulate partially bound to a protein ([growth hormone-binding protein](#), GHBP), which is the truncated part of the [growth hormone receptor](#), and an acid-labile subunit (ALS).

## Regulation

Secretion of growth hormone (GH) in the pituitary is regulated by the [neurosecretory](#) nuclei of the [hypothalamus](#). These cells release the peptides [Growth hormone-releasing hormone](#) (GHRH or *somatocrinin*) and [Growth hormone-inhibiting hormone](#) (GHIH or *somatostatin*) into the [hypophyseal portal](#) venous blood surrounding the pituitary. GH release in the pituitary is primarily determined by the balance of these two peptides, which in turn is affected by many physiological stimulators (e.g., exercise, nutrition, sleep) and inhibitors (e.g., free fatty acids) of GH secretion.<sup>[12]</sup>

[Somatotrophic cells](#) in the [anterior pituitary](#) gland then synthesize and secrete GH in a pulsatile manner, in response to these stimuli by the hypothalamus. The largest and most predictable of these GH peaks occurs about an hour after onset of sleep with plasma levels of 13 to 72 ng/mL.<sup>[13]</sup> Otherwise there is wide variation between days and individuals. Nearly fifty percent of GH secretion occurs during the third and fourth [NREM sleep](#) stages.<sup>[14]</sup> Surges of secretion during the day occur at 3- to 5-hour intervals.<sup>[3]</sup> The plasma concentration of GH during these peaks may range from 5 to even 45 ng/mL.<sup>[15]</sup> Between the peaks, basal GH levels are low, usually less than 5 ng/mL for most of the day and night.<sup>[13]</sup> Additional analysis of the pulsatile profile of GH described in all cases less than 1 ng/ml for basal levels while maximum peaks were situated around 10-20 ng/mL.<sup>[16][17]</sup>

A number of factors are known to affect GH secretion, such as age, sex, diet, exercise, stress, and other hormones.<sup>[3]</sup> Young adolescents secrete GH at the rate of about 700

µg/day, while healthy adults secrete GH at the rate of about 400 µg/day.<sup>[18]</sup> Sleep deprivation generally suppresses GH release, particularly after early adulthood.<sup>[19]</sup>

Stimulators of growth hormone (GH) secretion include:

- peptide hormones
  - [GHRH](#) (*somatocrinin*) through binding to the growth hormone-releasing hormone receptor ([GHRHR](#))<sup>[20]</sup>
  - [ghrelin](#) through binding to growth hormone secretagogue receptors ([GHSR](#))<sup>[21]</sup>
- sex hormones<sup>[22]</sup>
  - increased [androgen](#) secretion during puberty (in males from testis and in females from adrenal cortex)
  - [estrogen](#)
- [clonidine](#) and [L-DOPA](#) by stimulating GHRH release<sup>[23]</sup>
- [α4β2 nicotinic agonists](#), including [nicotine](#), which also act synergistically with [clonidine](#).<sup>[24][25][26]</sup>
- [hypoglycemia](#), [arginine](#)<sup>[27]</sup> and [propranolol](#) by inhibiting [somatostatin](#) release<sup>[23]</sup>
- [deep sleep](#)
- [niacin](#) as nicotinic acid (Vitamin B3)
- [fasting](#)
- vigorous [exercise](#) (and exercise in heat)
- heat exposure

*Inhibitors* of GH secretion include:

- [GHIH](#) (*somatostatin*) from the [periventricular nucleus](#)<sup>[32]</sup>
- circulating concentrations of GH and [IGF-1](#) ([negative feedback](#) on the pituitary and [hypothalamus](#))<sup>[3]</sup>
- [hyperglycemia](#)<sup>[23]</sup>
- [glucocorticoids](#)<sup>[33]</sup>
- [dihydrotestosterone](#)

In addition to control by endogenous and stimulus processes, a number of foreign compounds ([xenobiotics](#) such as drugs and [endocrine disruptors](#)) are known to influence GH secretion and function.<sup>[34]</sup>

## Function

Effects of growth hormone on the tissues of the body can generally be described as [anabolic](#) (building up). Like most other protein hormones, GH acts by interacting with a specific [receptor](#) on the surface of cells.

Increased height during childhood is the most widely known effect of GH. Height appears to be stimulated by at least two mechanisms:

1. Because [polypeptide](#) hormones are not fat-[soluble](#), they cannot penetrate [cell membranes](#). Thus, GH exerts some of its effects by binding to receptors on target cells, where it activates the [MAPK/ERK pathway](#).<sup>[35]</sup> Through this mechanism GH directly stimulates division and multiplication of [chondrocytes](#) of [cartilage](#).
2. GH also stimulates, through the [JAK-STAT signaling pathway](#),<sup>[35]</sup> the production of [insulin-like growth factor 1](#) (IGF-1, formerly known as somatomedin C), a hormone homologous to [proinsulin](#).<sup>[36]</sup> The [liver](#) is a major target organ of GH for this process and



is the principal site of IGF-1 production. IGF-1 has growth-stimulating effects on a wide variety of tissues. Additional IGF-1 is generated within target tissues, making it what appears to be both an [endocrine](#) and an [autocrine/paracrine](#) hormone. IGF-1 also has stimulatory effects on [osteoblast](#) and [chondrocyte](#) activity to promote bone growth.

In addition to increasing height in children and adolescents, growth hormone has many other effects on the body:

- Increases [calcium](#) retention, and strengthens and increases the [mineralization of bone](#)
- Increases [muscle](#) mass through [sarcomere hypertrophy](#)
- Promotes [lipolysis](#)
- Increases [protein synthesis](#)
- Stimulates the growth of all internal organs excluding the [brain](#)
- Plays a role in [homeostasis](#)
- Reduces [liver](#) uptake of [glucose](#)
- Promotes [gluconeogenesis](#) in the liver<sup>[37]</sup>
- Contributes to the maintenance and function of [pancreatic islets](#)
- Stimulates the [immune system](#)
- Increases deiodination of T4 to T3<sup>[38]</sup>

## Clinical significance

### Excess

The most common disease of GH excess is a pituitary tumor composed of somatotroph cells of the anterior pituitary. These somatotroph [adenomas](#) are benign and grow slowly, gradually producing more and more GH. For years, the principal clinical problems are those of GH excess. Eventually, the adenoma may become large enough to cause headaches, impair vision by pressure on the optic nerves, or cause deficiency of other pituitary hormones by displacement.

Prolonged GH excess thickens the bones of the jaw, fingers and toes. Resulting heaviness of the jaw and increased size of digits is referred to as [acromegaly](#). Accompanying problems can include sweating, pressure on nerves (e.g., [carpal tunnel syndrome](#)), muscle weakness, excess [sex hormone-binding globulin](#) (SHBG), insulin resistance or even a rare form of [type 2 diabetes](#), and reduced sexual function.

GH-secreting tumors are typically recognized in the fifth decade of life. It is extremely rare for such a tumor to occur in childhood, but, when it does, the excessive GH can cause excessive growth, traditionally referred to as [pituitary gigantism](#).

Surgical removal is the usual treatment for GH-producing tumors. In some circumstances, focused radiation or a GH antagonist such as [pegvisomant](#) may be employed to shrink the tumor or block function. Other drugs like [octreotide](#) (somatostatin agonist) and [bromocriptine](#) (dopamine agonist) can be used to block GH secretion because both somatostatin and dopamine negatively inhibit GHRH-mediated GH release from the anterior pituitary.<sup>[[citation needed](#)]</sup>

### Deficiency

Main article: [Growth hormone deficiency](#)

The effects of [growth hormone deficiency](#) vary depending on the age at which they occur. In children, [growth failure](#) and [short stature](#) are the major manifestations of GH deficiency, with common causes including genetic conditions and congenital malformations. It can also cause delayed sexual maturity. In adults, deficiency is rare,<sup>[39]</sup> with the most common cause a pituitary adenoma, and others including a continuation of a childhood problem, other structural lesions or trauma, and very rarely idiopathic GHD.

Adults with GHD "tend to have a relative increase in fat mass and a relative decrease in muscle mass and, in many instances, decreased energy and quality of life".<sup>[39]</sup>

Diagnosis of GH deficiency involves a multiple-step diagnostic process, usually culminating in GH stimulation tests to see if the patient's pituitary gland will release a pulse of GH when provoked by various stimuli.

## Psychological effects

### Quality of life

Several studies, primarily involving patients with [GH deficiency](#), have suggested a crucial role of GH in both mental and emotional well-being and maintaining a high energy level. Adults with GH deficiency often have higher rates of [depression](#) than those without.<sup>[40]</sup> While [GH replacement therapy](#) has been proposed to treat depression as a result of GH deficiency, the long-term effects of such therapy are unknown.<sup>[40]</sup>

### Cognitive function

GH has also been studied in the context of [cognitive function](#), including learning and memory.<sup>[41]</sup> GH in humans appears to induce cognitive function and may be useful in the treatment of patients with cognitive impairment that is a result of GH deficiency.<sup>[41]</sup>

## Medical uses

Main article: [Growth hormone treatment](#)

### Replacement therapy

Treatment with exogenous GH is indicated only in limited circumstances,<sup>[39]</sup> and needs regular monitoring due to the frequency and severity of side-effects. GH is used as replacement therapy in adults with GH deficiency of either childhood-onset or adult-onset (usually as a result of an acquired pituitary tumor). In these patients, benefits have variably included reduced fat mass, increased lean mass, increased bone density, improved lipid profile, reduced cardiovascular risk factors, and improved psychosocial well-being.

### Other approved uses

GH can be used to treat conditions that produce short stature but are not related to deficiencies in GH. However, results are not as dramatic when compared to short

stature that is solely attributable to deficiency of GH. Examples of other causes of shortness often treated with GH are [Turner syndrome](#), [chronic renal failure](#), [Prader–Willi syndrome](#), [intrauterine growth restriction](#), and severe [idiopathic short stature](#). Higher ("pharmacologic") doses are required to produce significant acceleration of growth in these conditions, producing blood levels well above normal ("physiologic"). Despite the higher doses, side-effects during treatment are rare, and vary little according to the condition being treated.

One version of rHGH has also been FDA approved for maintaining muscle mass in [wasting](#) due to [AIDS](#).<sup>[42]</sup>

#### Off-label use

Main article: [HGH controversies](#)

[Off-label](#) prescribing of HGH is controversial and may be illegal.

Claims for GH as an anti-aging treatment date back to 1990 when the *New England Journal of Medicine* published a study wherein GH was used to treat 12 men over 60.<sup>[43]</sup> At the conclusion of the study, all the men showed statistically significant increases in lean body mass and bone mineral density, while the control group did not. The authors of the study noted that these improvements were the opposite of the changes that would normally occur over a 10- to 20-year aging period. Despite the fact the authors at no time claimed that GH had reversed the aging process itself, their results were misinterpreted as indicating that GH is an effective anti-aging agent.<sup>[44][45][46]</sup> This has led to organizations such as the controversial [American Academy of Anti-Aging Medicine](#) promoting the use of this hormone as an "anti-aging agent".<sup>[47]</sup>

A Stanford University School of Medicine [meta-analysis](#) of clinical studies on the subject published in early 2007 showed that the application of GH on healthy elderly patients increased muscle by about 2 kg and decreased body fat by the same amount.<sup>[44]</sup> However, these were the only positive effects from taking GH. No other critical factors were affected, such as bone density, cholesterol levels, lipid measurements, maximal oxygen consumption, or any other factor that would indicate increased fitness.<sup>[44]</sup> Researchers also did not discover any gain in muscle strength, which led them to believe that GH merely let the body store more water in the muscles rather than increase muscle growth. This would explain the increase in lean body mass.

GH has also been used experimentally to treat [multiple sclerosis](#), to enhance [weight loss](#) in [obesity](#), as well as in [fibromyalgia](#), [heart failure](#), [Crohn's disease](#) and [ulcerative colitis](#), and burns. GH has also been used experimentally in patients with [short bowel syndrome](#) to lessen the requirement for intravenous [total parenteral nutrition](#).

In 1990, the US Congress passed an omnibus crime bill, the [Crime Control Act of 1990](#), that amended the [Federal Food, Drug, and Cosmetic Act](#), that classified [anabolic steroids](#) as [controlled substances](#) and added a new section that stated that a person who "knowingly distributes, or possesses with intent to distribute, human growth hormone for any use in humans other than the treatment of a disease or other recognized medical condition, where such use has been authorized by the Secretary of Health and Human Services" has committed a [felony](#).<sup>[48][49]</sup>

The [Drug Enforcement Administration](#) of the US Department of Justice considers off-label prescribing of HGH to be illegal, and to be a key path for illicit distribution of HGH.<sup>[50]</sup> This section has also been interpreted by some doctors, most notably<sup>[48][51]</sup> the authors of a commentary article published in the [Journal of the American Medical Association](#) in 2005, as meaning that *prescribing* HGH off-label may be considered illegal.<sup>[52]</sup> And some articles in the popular press, such as those criticizing the pharmaceutical industry for marketing drugs for off-label use (which is clearly illegal) have made strong statements about whether doctors can prescribe HGH off-label: "Unlike other prescription drugs, HGH may be prescribed only for specific uses. U.S. sales are limited by law to treat a rare growth defect in children and a handful of uncommon conditions like short bowel syndrome or Prader-Willi syndrome, a congenital disease that causes reduced muscle tone and a lack of hormones in sex glands."<sup>[53][54]</sup> At the same time, anti-aging clinics where doctors prescribe, administer, and sell HGH to people are big business.<sup>[53][55]</sup> In a 2012 article in Vanity, Fair, when asked how HGH prescriptions far exceed the number of adult patients estimated to have HGH-deficiency, Dr. Dragos Roman, who leads a team at the FDA that reviews drugs in endocrinology, said "The F.D.A. doesn't regulate off-label uses of H.G.H. Sometimes it's used appropriately. Sometimes it's not."<sup>[55]</sup>

### Side-effects

Use of GH as a drug has been approved by the FDA for several indications. This means that the drug has acceptable safety in light of its benefits when used in the approved way. Like every drug, there are several side effects caused by GH, some common, some rare. Injection-site reaction is common. More rarely, patients can experience joint swelling, joint pain, [carpal tunnel syndrome](#), and an increased risk of [diabetes](#).<sup>[44]</sup> In some cases, the patient can produce an immune response against GH. GH may also be a risk factor for [Hodgkin's lymphoma](#).<sup>[56]</sup>

One survey of adults that had been treated with replacement cadaver GH (which has not been used anywhere in the world since 1985) during childhood showed a mildly increased incidence of colon cancer and prostate cancer, but linkage with the GH treatment was not established.<sup>[57]</sup>

### Performance enhancement

The first description of the use of GH as a doping agent was Dan Duchaine's "Underground Steroid handbook" which emerged from California in 1982; it is not known where and when GH was first used this way. Athletes in many sports have used human growth hormone in order to attempt to enhance their athletic performance. Some recent studies have not been able to support claims that human growth hormone can improve the athletic performance of professional male athletes. Many athletic societies ban the use of GH and will issue sanctions against athletes who are caught using it. In the United States, GH is legally available only by prescription from a medical doctor.

### Dietary supplements

To capitalize on the idea that GH might be useful to combat aging, companies selling [dietary supplements](#) have websites selling products linked to GH in the advertising text,

with medical-sounding names described as "HGH Releasers". Typical ingredients include amino acids, minerals, vitamins, and/or herbal extracts, the combination of which are described as causing the body to make more GH with corresponding beneficial effects. In the United States, because these products are marketed as dietary supplements it is illegal for them to contain GH, which is a drug. Also, under United States law, products sold as dietary supplements cannot have claims that the supplement treats or prevents any disease or condition, and the advertising material must contain a statement that the health claims are not approved by the FDA. The FTC and the FDA do enforce the law when they become aware of violations.<sup>[62]</sup>

## Heat Shock Proteins (HSPs)

Oxidative stress is a primary cause of protein degradation. Anything which can help prevent or minimize oxidative protein damage, or help repair damaged proteins while maintaining protein synthesis, will result in a net increase in protein synthesis and help build muscle tissue. Heat shock proteins (HSPs) are induced by heat and other stressors including vigorous exercise, including exposure to cold, UV light, wound healing and tissue remodeling. Numerous studies have shown that intermittent exposure to heat induces a protective stress response which promotes the expression of the heat shock factor 1 gene and HSPs which help the body resist stress. HSPs have many beneficial functions including the following:

1. **Chaperone functions:** Some HSPs, called “chaperones”, ensure that cell proteins are in the correct shape and in the right place at the right time. HSPs help new or damaged and misfolded proteins to fold into their correct three-dimensional conformations, which is essential for their function. HSPs also shuttle proteins from one compartment to another inside the cell, and target old or terminally misfolded proteins to proteases for degradation.
2. **Antioxidant functions:** HSPs help prevent damage by scavenging free radicals and supporting cellular antioxidant capacities.
3. **Prevention of muscle protein degradation:** It is also believed that one specific HSP known as HSP72 plays a protective role in the prevention of muscle protein degradation during periods of reduced contractile activity. A conceivable link between HSP72 and reduced protein degradation in muscle is based on evidence demonstrating that muscle atrophy induced by immobilization is associated with oxidative injury in myocytes.

**Peptides** are naturally occurring biological molecules. They are short chains of [amino acid monomers](#) linked by [peptide \(amide\)](#) bonds. The [covalent chemical bonds](#) are formed when the [carboxyl](#) group of one amino acid reacts with the [amino](#) group of another. The shortest peptides are [dipeptides](#), consisting of 2 amino acids joined by a single peptide bond, followed by [tripeptides](#), [tetrapeptides](#), etc. A **polypeptide** is a long, continuous, and unbranched peptide chain. Hence, peptides fall under the broad chemical classes of biological [oligomers](#) and [polymers](#), alongside [nucleic acids](#), [oligosaccharides](#) and [polysaccharides](#), etc.

Peptides are distinguished from [proteins](#) on the basis of size, and as an arbitrary benchmark can be understood to contain approximately 50 or fewer amino acids.<sup>[1]</sup> Proteins consist of one or more polypeptides arranged in a biologically functional way, often bound to [ligands](#) such as [coenzymes](#) and [cofactors](#), or to another protein or other [macromolecule](#) ([DNA](#), [RNA](#), etc.), or to complex [macromolecular assemblies](#). Finally, while aspects of the techniques that apply to peptides *versus* polypeptides and proteins differ (i.e., in the specifics of [electrophoresis](#), [chromatography](#), etc.), the size boundaries that distinguish peptides from polypeptides and proteins are not absolute: long peptides such as [amyloid beta](#) have been referred to as proteins, and smaller proteins like [insulin](#) have been considered peptides.

Amino acids that have been incorporated into peptides are termed "residues" due to the release of either a hydrogen ion from the amine end or a [hydroxyl](#) ion from the carboxyl



end, or both, as a water molecule is released during formation of each amide bond.<sup>[2]</sup> All peptides except [cyclic peptides](#) have an [N-terminal](#) and [C-terminal](#) residue at the end of the peptide (as shown for the tetrapeptide in the image).

**Heat shock proteins (HSP)** are a family of proteins that are produced by [cells](#) in response to exposure to stressful conditions. They were first described in relation to [heat shock](#). **Heat shock** is the effect of subjecting a cell to a higher temperature than that of the ideal [body temperature](#) of the organism from which the [cell line](#) was derived. The cellular response to heat shock includes the transcriptional up-regulation of genes encoding [heat shock proteins](#) (HSPs) as part of the [cell](#)'s internal repair mechanism.<sup>[1]</sup> They are also called stress-proteins.<sup>[2]</sup> and respond to heat, cold and [oxygen](#) deprivation by activating several [cascade pathways](#). HSPs are also present in cells under perfectly normal conditions.<sup>[2]</sup> Some HSPs, called [chaperones](#), ensure that the cell's proteins are in the right shape and in the right place at the right time.<sup>[1][2]</sup> For example, HSPs help new or misfolded proteins to fold into their correct three-dimensional conformations, which is essential for their function.<sup>[2]</sup> They also shuttle proteins from one compartment to another inside the cell, and target old or terminally misfolded proteins to [proteases](#) for degradation.<sup>[2]</sup> Heat shock proteins are also believed to play a role in the presentation of pieces of proteins (or [peptides](#)) on the cell surface to help the [immune system](#) recognize diseased cells.<sup>[3]</sup>

The up-regulation of HSPs during heat shock is generally controlled by a single [transcription factor](#); in eukaryotes this regulation is performed by [heat shock factor](#) (HSF), while  $\sigma^{32}$  is the heat shock [sigma factor](#) in *Escherichia coli*.<sup>[1]</sup>

In [molecular biology](#), **molecular chaperones** are [proteins](#) that assist the covalent folding or unfolding and the assembly or disassembly of other macromolecular structures. Chaperones are present when the macromolecules perform their normal biological functions and have correctly completed the processes of folding and/or assembly. The chaperones are concerned primarily with protein folding. The first protein to be called a chaperone assists the assembly of [nucleosomes](#) from folded [histones](#) and [DNA](#) and such assembly chaperones, especially in the nucleus,<sup>[1][2]</sup> are concerned with the assembly of folded subunits into oligomeric structures.<sup>[3]</sup>

One major function of chaperones is to prevent both newly synthesised polypeptide chains and assembled subunits from aggregating into nonfunctional structures. It is for this reason that many chaperones, but by no means all, are [heat shock proteins](#) because the tendency to aggregate increases as proteins are denatured by stress. In this case, chaperones do not convey any additional [steric](#) information required for proteins to fold. However, some highly specific 'steric chaperones' do convey unique structural (steric) information onto proteins, which cannot be folded spontaneously. Such proteins violate [Anfinsen's dogma](#).<sup>[4]</sup>

Various approaches have been applied to study the structure, dynamics and functioning of chaperones. Bulk biochemical measurements have informed us on the protein folding efficiency, and prevention of aggregation when chaperones are present during protein folding. Recent advances in single-molecule analysis<sup>[5]</sup> have brought insights into structural heterogeneity of chaperones, folding intermediates and affinity of chaperones for unstructured and structured protein chains.

This increase in expression is [transcriptionally](#) regulated. The dramatic [upregulation](#) of the heat shock proteins is a key part of the [heat shock response](#) and is induced primarily by [heat shock factor](#) (HSF).<sup>[6]</sup> HSPs are found in virtually all living organisms, from [bacteria](#) to [humans](#).

Heat-shock proteins are named according to their molecular weight. For example, [Hsp60](#), [Hsp70](#) and [Hsp80](#) (the most widely-studied HSPs) refer to families of heat shock proteins on the order of 60, 70, and 80 [kilodaltons](#) in size, respectively.<sup>[7]</sup> The small 8-kilodalton protein [ubiquitin](#), which marks proteins for degradation, also has features of a heat shock protein.<sup>[8]</sup>

## Discovery

It is known that rapid heat hardening can be elicited by a brief exposure of cells to sub-lethal high temperature, which in turn provides protection from subsequent and more severe temperature. In 1962, Italian geneticist [Ferruccio Ritossa](#) reported that heat and the metabolic uncoupler [2,4-dinitrophenol](#) induced a characteristic pattern of puffing in the [chromosomes](#) of [Drosophila](#).<sup>[9][10]</sup> This discovery eventually led to the identification of the heat-shock proteins (HSP) or stress proteins whose expression these puffs represented. Increased synthesis of selected proteins in *Drosophila* cells following stresses such as heat shock was first reported in 1974.<sup>[11]</sup>

Beginning in the mid-1960s, investigators recognized that many HSPs function as molecular [chaperones](#) and thus play a critical role in protein folding, intracellular trafficking of proteins, and coping with proteins denatured by heat and other stresses. Therefore, the study of stress proteins has undergone explosive growth.

## Function

### Upregulation in stress

Production of high levels of heat shock proteins can also be triggered by exposure to different kinds of environmental [stress](#) conditions, such as [infection](#), [inflammation](#), exercise, exposure of the cell to toxins ([ethanol](#), [arsenic](#), [trace metals](#), and [ultraviolet](#) light, among many others), [starvation](#), [hypoxia](#) ([oxygen](#) deprivation), nitrogen deficiency (in plants), or water deprivation. As a consequence, the heat shock proteins are also referred to as **stress proteins** and their [upregulation](#) is sometimes described more generally as part of the **stress response**.

The mechanism by which heat-shock (or other environmental stressors) activates the heat shock factor has been determined in bacteria. During heat stress outer membrane proteins (OMPs) do not fold and cannot insert correctly into the outer membrane. They accumulate in the periplasmic space. These OMP's are detected by DegS, an inner membrane protease, that passes the signal through the membrane to the sigmaE transcription factor.<sup>[13]</sup> However, some studies suggest that an increase in damaged or abnormal proteins brings HSPs into action.



Some bacterial heat shock proteins are upregulated via a mechanism involving RNA thermometers such as the [FourU thermometer](#), [ROSE element](#) and the [Hsp90 cis-regulatory element](#).<sup>[14]</sup>

### Role as chaperone

Several heat shock proteins function as intra-cellular [chaperones](#) for other proteins. They play an important role in protein-protein interactions such as folding and assisting in the establishment of proper protein [conformation](#) (shape) and prevention of unwanted protein aggregation. By helping to stabilize partially unfolded proteins, HSPs aid in transporting proteins across membranes within the cell.<sup>[15][16]</sup>

Some members of the HSP family are expressed at low to moderate levels in *all* organisms because of their essential role in protein maintenance.

### Management

Heat-shock proteins also occur under non-stressful conditions, simply "monitoring" the cell's proteins. Some examples of their role as "monitors" are that they carry old proteins to the cell's "recycling bin" ([proteasome](#)) and they help newly synthesised proteins fold properly.

These activities are part of a cell's own repair system, called the "cellular stress response" or the "heat-shock response".

### Cardiovascular

Heat shock proteins appear to serve a significant cardiovascular role. Hsp90, [hsp84](#), hsp70, [hsp27](#), [hsp20](#), and [alpha B crystallin](#) all have been reported as having roles in the cardiovascular system.<sup>[17]</sup>

[Hsp90](#) binds both [endothelial nitric oxide synthase](#) and soluble [guanylate cyclase](#), which in turn are involved in vascular relaxation.<sup>[18]</sup>

A kinase of the nitric oxide cell signalling pathway, [protein kinase G](#), phosphorylates a small heat shock protein, hsp20. Hsp20 phosphorylation correlates well with smooth muscle relaxation and is one significant phosphoprotein involved in the process.<sup>[19]</sup> Hsp20 appears significant in development of the smooth muscle phenotype during development. Hsp20 also serves a significant role in preventing platelet aggregation, cardiac myocyte function and prevention of apoptosis after ischemic injury, and skeletal muscle function and muscle insulin response.<sup>[20]</sup>

Hsp27 is a major phosphoprotein during women's contractions. Hsp27 functions in small muscle migrations and appears to serve an integral role.<sup>[21]</sup>

### Immunity

Extracellular and membrane bound heat-shock proteins, especially [Hsp70](#) are involved in binding [antigens](#) and presenting them to the [immune system](#).<sup>[22]</sup>

## Clinical significance

Heat Shock Factor 1 ([HSF1](#)) is a transcription factor that is involved in the general maintenance and upregulation of Hsp70 protein expression.<sup>[23] [24]</sup> Recently it was discovered that HSF1 is a powerful multifaceted modifier of [carcinogenesis](#). HSF1 [knockout mice](#) show significantly decreased incidence of skin tumor after topical application of [DMBA](#) (7,12-dimethylbenzanthracene), a [mutagen](#).<sup>[25]</sup> Moreover, HSF1 inhibition by a potent RNA [aptamer](#) attenuates mitogenic (MAPK) signaling and induces cancer cell [apoptosis](#).<sup>[26]</sup>

## Applications

### Prevention of Muscle Protein Degradation and Muscle Repair

It also seems possible that HSP72 can play a protective role in the prevention of muscle protein degradation during periods of reduced contractile activity. A conceivable link between HSP72 and reduced protein degradation in muscle is as follows. Recent evidence demonstrates that muscle atrophy induced by immobilization is associated with oxidative injury in myocytes. This increase in oxidative stress may accelerate muscle protein breakdown because oxidatively modified proteins are more susceptible to proteolytic attack. Indeed, numerous oxidative modifications of proteins are concomitant with elevated proteolysis (5). In this regard, a function of HSP72 is to bind to nonnative or misfolded proteins and prevent their aggregation by promotion of refolding or renaturation (9). Hence, it seems conceivable that high relative levels of HSP72 could reduce the rate of proteolysis in cells during oxidative stress by repair of damaged proteins. Therefore, on the basis of the collective links between HSP72 and protein synthesis/degradation, it is conceivable that elevating HSP72 in skeletal muscle before unloading could be a countermeasure to retard disuse-induced muscle atrophy. Therefore, the present study was performed to test the hypothesis that exposure to whole body heat stress before unloading of skeletal muscle would elevate muscle levels of HSP72 and attenuate the muscle atrophy associated with short-term, hindlimb-unloading in rats.

### Cancer vaccine adjuvant

Given their role in [antigen presentation](#),<sup>[22]</sup> HSPs are useful as [immunologic adjuvants](#) in boosting the response to a [vaccine](#).<sup>[27]</sup> Furthermore, some researchers speculate that HSPs may be involved in binding protein fragments from dead malignant cells and presenting them to the immune system.<sup>[28]</sup> Therefore HSPs may be useful for increasing the effectiveness of cancer vaccines.<sup>[22][29]</sup>

### Anticancer therapeutics

Intracellular heat shock proteins are highly expressed in cancerous cells and are essential to the survival of these cell types. Hence [small molecule inhibitors of HSPs](#), especially [Hsp90](#) show promise as anticancer agents.<sup>[30]</sup> The potent Hsp90 inhibitor [17-AAG](#) is currently in [clinical trials](#) for the treatment of several types of cancer.<sup>[31]</sup>

HSPgp96 also shows promise as an anticancer treatment and is currently in clinical trials against non-small cell lung cancer.<sup>[32]</sup>

### Agricultural

Researchers are also investigating the role of HSPs in conferring stress tolerance to hybridized plants, hoping to address drought and poor soil conditions for farming.<sup>[33]</sup>

The principal heat-shock proteins that have chaperone activity belong to five conserved classes: [HSP33](#), [HSP60](#), [HSP70](#), [HSP90](#), [HSP100](#), and the small heat-shock proteins ([sHSPs](#)).<sup>[11]</sup>

## THE FOXO3 GENE

In addition to HSPs, FOXO3 is another molecular pathway that may explain how heat exposure can improve longevity. *Foxo3* is a gene that is associated with longevity. FoxO3, one of the four mammalian FoxO genes, is activated by heat stress. Humans with a polymorphism that makes more of *foxo3* have up to a 2.7fold increased chance of living to be a centenarian and In mice, having more of their homologous version of this gene can extend their lifespan by up to 30% !

The mechanism by which FOXO3 increases longevity has to do with the fact that it is a master regulator of many different genes. When it is on, it increases the expression of several genes that make you [more resilient various types of stress that occur with aging](#). Many of the genes that FOXO3 increases happen to decrease with age, so it is good to boost their expression.

One particularly important type of stress that FOXO3 protects against is DNA damage. The same type of reactive byproducts (from normal metabolism and immune function) that damage proteins in the cell also damage DNA. [DNA damage can lead to mutations](#) and a damaged cell with a mutation may then replicate to form cancer. [Foxo3 increases the expression of DNA repair genes that repair that damage to DNA](#) so that a mutation never occurs. It also increases the expression of genes that kill cell damaged cells so that they do not become cancer cell. FOXO3 also makes cells more resilient to damage by increasing the expression of genes that combat this damage [including antioxidant genes](#) (which are much more potent than dietary antioxidants) and prevent the damage from reaching the cell. When a cell becomes damaged or its telomeres become critically short, the cell can become senescent (which means the cell does not die but it is not alive either) and it just sits around causing more damage because a senescent cell releases proinflammatory cytokines and other factors that damage more cells. Well, [FOXO3 increases genes involved in autophagy](#) , which means the cell will eat itself up so that it is not secreting inflammatory molecules that damage more cells. FOXO3 also increases the expression [genes involved in immune function](#) (which declines with age) so that your immune cells can fight off bacteria, viruses and cancer cells better. FOXO3 also regulates genes involved in metabolism and stem cell function just to name a few!

*DNA damage occurs everyday and can lead to breaks in both DNA strands (called doublestrand breaks). This type of DNA damage is very dangerous because it is the most difficult to repair and leads to mutation that are known to cause cancer. Heat stress activates FOXO3, which increases the production of genes that produce DNA repair enzymes to repair this damage so a cancer causing mutation does not occur.*

### The pro-longevity gene FoxO3 is a direct target of the p53 tumor suppressor

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#### Abstract

FoxO transcription factors have a conserved role in longevity, and act as tissue-specific tumor suppressors in mammals. Several nodes of interaction have been identified between FoxO transcription factors and p53, a major tumor suppressor in humans and mice. However, the extent and importance of the functional interaction between FoxO and p53 have not been fully

explored. Here, we show that p53 regulates the expression of FoxO3, one of the four mammalian FoxO genes, in response to DNA damaging agents in both mouse embryonic fibroblasts and thymocytes. We find that p53 transactivates FoxO3 in cells by binding to a site in the second intron of the FoxO3 gene, a genomic region recently found to be associated with extreme longevity in humans. While FoxO3 is not necessary for p53-dependent cell cycle arrest, FoxO3 appears to modulate p53-dependent apoptosis. We also find that FoxO3 loss does not interact with p53 loss for tumor development in vivo, although the tumor spectrum of p53-deficient mice appears to be affected by FoxO3 loss. Our findings indicate that FoxO3 is a p53 target gene, and suggest that FoxO3 and p53 are part of a regulatory transcriptional network that may have an important role during aging and cancer.

Aging is regulated by modifications in single genes and by simple changes in the environment. The signaling pathway connecting insulin to FoxO transcription factors integrates environmental stimuli to regulate lifespan. FoxO transcription factors are directly phosphorylated in response to insulin/growth factor signaling by the protein kinase Akt, thereby causing their sequestration in the cytoplasm. In the absence of insulin/growth factors, FoxO factors translocate to the nucleus where they trigger a range of cellular responses, including resistance to oxidative stress—a phenotype highly coupled with lifespan extension. Our recent results indicate that FoxO transcription factors are also regulated in response to nutrient deprivation by the AMP-activated protein kinase (AMPK) pathway. The energy-sensing AMPK directly phosphorylates FoxO transcription factors at six regulatory sites. AMPK phosphorylation enhances FoxO transcriptional activity, leading to the expression of specific target genes involved in stress resistance and changes in energy metabolism. The AMPK–FoxO pathway plays a crucial role in the ability of a dietary restriction regimen to extend lifespan in *Caenorhabditis elegans*. Understanding the intricate signaling networks that translate environmental conditions like dietary restriction into changes in gene expression that extend lifespan will be of critical importance to identify ways to delay the onset of aging and age-dependent diseases.

# Mitochondrion

From Wikipedia, the free encyclopedia

The **mitochondrion** (plural **mitochondria**) is a [membrane-bound organelle](#) found in most [eukaryotic cells](#).<sup>[1]</sup> The word mitochondrion comes from the [Greek](#) *μίτος*, *mitos*, i.e. "thread", and *χονδρίον*, *chondrion*, i.e. "granule"<sup>[2]</sup> or "grain-like".

Mitochondria range from 0.5 to 1.0 [μm](#) in diameter. These structures are sometimes described as "the powerhouse of the cell" because they generate most of the cell's supply of [adenosine triphosphate](#) (ATP), used as a source of [chemical energy](#).<sup>[3]</sup> In addition to supplying cellular energy, mitochondria are involved in other tasks such as [signaling](#), [cellular differentiation](#), [cell death](#), as well as maintaining the control of the [cell cycle](#) and [cell growth](#).<sup>[4]</sup> Mitochondria have been implicated in several human diseases, including [mitochondrial disorders](#),<sup>[5]</sup> [cardiac](#) dysfunction,<sup>[6]</sup> and heart failure. A recent University of California study including ten children diagnosed with severe autism suggests that [autism](#) may be correlated with mitochondrial defects as well.<sup>[7]</sup>

Several characteristics make mitochondria unique. The number of mitochondria in a cell can vary widely by [organism](#), [tissue](#), and cell type. For instance, [red blood cells](#) have no mitochondria, whereas [liver cells](#) can have more than 2000.<sup>[8][9]</sup> The organelle is composed of compartments that carry out specialized functions. These compartments or regions include the outer membrane, the [intermembrane space](#), the [inner membrane](#), and the [cristae](#) and [matrix](#). Mitochondrial proteins vary depending on the tissue and the species. In humans, 615 distinct types of protein have been identified from [cardiac](#) mitochondria,<sup>[10]</sup> whereas in [rats](#), 940 proteins have been reported.<sup>[11]</sup> The mitochondrial [proteome](#) is thought to be dynamically regulated.<sup>[12]</sup> Although most of a cell's [DNA](#) is contained in the [cell nucleus](#), the mitochondrion has its own independent [genome](#). Further, its DNA shows substantial similarity to [bacterial genomes](#).<sup>[13]</sup>

## History

The first observations of intracellular structures that probably represent mitochondria were published in the 1840s.<sup>[14]</sup> [Richard Altmann](#), in 1894, established them as cell organelles and called them "bioblasts".<sup>[14]</sup> The term "mitochondria" itself was coined by Carl Benda in 1898.<sup>[14]</sup> [Lemon Michaelis](#) discovered that [Janus green](#) can be used as a [supravital stain](#) for mitochondria in 1900. [Friedrich Meves](#), in 1904, made the first recorded observation of mitochondria in plants (*Nymphaea alba*)<sup>[14][15]</sup> and in 1908, along with [Claudius Regaud](#), suggested that they contain proteins and lipids. Benjamin F. Kingsbury, in 1912, first related them with cell respiration, but almost exclusively based on morphological observations.<sup>[14]</sup> In 1913 particles from extracts of guinea-pig liver were linked to respiration by [Otto Heinrich Warburg](#), which he called "grana". Warburg and [Heinrich Otto Wieland](#), who had also postulated a similar particle mechanism, disagreed on the chemical nature of the respiration. It was not until 1925 when [David Keilin](#) discovered [cytochromes](#) that the [respiratory chain](#) was described.<sup>[14]</sup>

In 1939, experiments using minced muscle cells demonstrated that cellular respiration using one oxygen atom can form two [adenosine triphosphate](#) (ATP) molecules, and, in 1941, the concept of the phosphate bonds of ATP being a form of energy in cellular metabolism was developed by [Fritz Albert Lipmann](#). In the following years, the

mechanism behind cellular respiration was further elaborated, although its link to the mitochondria was not known.<sup>[14]</sup> The introduction of [tissue fractionation](#) by [Albert Claude](#) allowed mitochondria to be isolated from other cell fractions and biochemical analysis to be conducted on them alone. In 1946, he concluded that [cytochrome oxidase](#) and other enzymes responsible for the respiratory chain were isolated to the mitochondria. Over time, the fractionation method was tweaked, improving the quality of the mitochondria isolated, and other elements of cell respiration were determined to occur in the mitochondria.<sup>[14]</sup>

The first high-resolution [micrographs](#) appeared in 1952, replacing the Janus Green stains as the preferred way of visualising the mitochondria. This led to a more detailed analysis of the structure of the mitochondria, including confirmation that they were surrounded by a membrane. It also showed a second membrane inside the mitochondria that folded up in ridges dividing up the inner chamber and that the size and shape of the mitochondria varied from cell to cell.

The popular term "powerhouse of the cell" was coined by Philip Siekevitz in 1957.<sup>[16]</sup>

In 1967, it was discovered that mitochondria contained [ribosomes](#). In 1968, methods were developed for mapping the mitochondrial genes, with the genetic and physical map of yeast mitochondria being completed in 1976.<sup>[14]</sup>

## Structure

**Mitochondrion ultrastructure** (*interactive diagram*) A mitochondrion has a double membrane; the inner one contains its [chemiosmotic](#) apparatus and has deep grooves which increase its surface area. While commonly depicted as an "orange sausage with a blob inside of it" (like it is here), mitochondria can take many shapes<sup>[17]</sup> and their intermembrane space is quite thin.

A mitochondrion contains outer and inner membranes composed of [phospholipid bilayers](#) and [proteins](#).<sup>[8]</sup> The two membranes have different properties. Because of this double-membraned organization, there are five distinct parts to a mitochondrion. They are:

1. the outer mitochondrial membrane,
2. the intermembrane space (the space between the outer and inner membranes),
3. the inner mitochondrial membrane,
4. the cristae space (formed by infoldings of the inner membrane), and
5. the matrix (space within the inner membrane).

Mitochondria stripped of their outer membrane are called [mitoplasts](#).

### Outer membrane

The **outer mitochondrial membrane**, which encloses the entire organelle, is 60 to 75 [angstroms](#) (Å) thick. It has a protein-to-phospholipid ratio similar to that of the eukaryotic plasma membrane (about 1:1 by weight). It contains large numbers of [integral membrane proteins](#) called [porins](#). These porins form channels that allow molecules of 5000 [daltons](#) or less in molecular weight to freely [diffuse](#) from one side of the membrane to the other.<sup>[8]</sup> Larger proteins can enter the mitochondrion if a signaling



sequence at their [N-terminus](#) binds to a large multisubunit [protein](#) called [translocase](#) of the outer membrane, which then [actively moves](#) them across the membrane.<sup>[18]</sup> Mitochondrial [pro-proteins](#) are imported through specialised translocate complexes. The outer membrane also contains [enzymes](#) involved in such diverse activities as the elongation of [fatty acids](#), [oxidation](#) of [epinephrine](#), and the [degradation](#) of [tryptophan](#). These enzymes include [monoamine oxidase](#), [rotenone](#)-insensitive NADH-cytochrome c-reductase, [kynurenine hydroxylase](#) and fatty acid Co-A [ligase](#). Disruption of the outer membrane permits proteins in the intermembrane space to leak into the cytosol, leading to certain cell death.<sup>[19]</sup> The mitochondrial outer membrane can associate with the endoplasmic reticulum (ER) membrane, in a structure called MAM (mitochondria-associated ER-membrane). This is important in the ER-mitochondria calcium signaling and is involved in the transfer of lipids between the ER and mitochondria.<sup>[20]</sup> Outside the outer membrane there are small (diameter: 60Å) particles named sub-units of Parson.

### Intermembrane space

The [intermembrane space](#) is the space between the outer membrane and the inner membrane. It is also known as perimitochondrial space. Because the outer membrane is freely permeable to small molecules, the concentrations of small molecules such as ions and sugars in the intermembrane space is the same as the [cytosol](#).<sup>[8]</sup> However, large proteins must have a specific signaling sequence to be transported across the outer membrane, so the protein composition of this space is different from the protein composition of the [cytosol](#). One protein that is localized to the intermembrane space in this way is [cytochrome c](#).<sup>[19]</sup>

### Inner membrane

Main article: [Inner mitochondrial membrane](#)

The inner mitochondrial membrane contains proteins with five types of functions:<sup>[8]</sup>

1. Those that perform the [redox](#) reactions of [oxidative phosphorylation](#)
2. [ATP synthase](#), which generates [ATP](#) in the matrix
3. Specific [transport proteins](#) that regulate [metabolite](#) passage into and out of the matrix
4. Protein import machinery
5. [Mitochondrial fusion](#) and [fission](#) protein

It contains more than 151 different [polypeptides](#), and has a very high protein-to-phospholipid ratio (more than 3:1 by weight, which is about 1 protein for 15 phospholipids). The inner membrane is home to around 1/5 of the total protein in a mitochondrion.<sup>[8]</sup> In addition, the inner membrane is rich in an unusual phospholipid, [cardiolipin](#). This phospholipid was originally discovered in [cow](#) hearts in 1942, and is usually characteristic of mitochondrial and bacterial plasma membranes.<sup>[21]</sup> Cardiolipin contains four fatty acids rather than two, and may help to make the inner membrane impermeable.<sup>[8]</sup> Unlike the outer membrane, the inner membrane doesn't contain porins, and is highly impermeable to all molecules. Almost all ions and molecules require special membrane transporters to enter or exit the matrix. Proteins are ferried into the matrix via the [translocase of the inner membrane](#) (TIM) complex or via Oxa1.<sup>[18]</sup> In addition, there is a membrane potential across the inner membrane, formed by the action of the enzymes of the [electron transport chain](#).



## Cristae

The inner mitochondrial membrane is compartmentalized into numerous [cristae](#), which expand the surface area of the inner mitochondrial membrane, enhancing its ability to produce ATP. For typical liver mitochondria, the area of the inner membrane is about five times as large as the outer membrane. This ratio is variable and mitochondria from cells that have a greater demand for ATP, such as muscle cells, contain even more cristae. These folds are studded with small round bodies known as [F<sub>1</sub> particles](#) or oxysomes. These are not simple random folds but rather invaginations of the inner membrane, which can affect overall [chemiosmotic](#) function.<sup>[22]</sup>

One recent mathematical modeling study has suggested that the optical properties of the cristae in filamentous mitochondria may affect the generation and propagation of light within the tissue.<sup>[23]</sup>

## Matrix

Main article: [Mitochondrial matrix](#)

The matrix is the space enclosed by the inner membrane. It contains about 2/3 of the total protein in a mitochondrion.<sup>[8]</sup> The matrix is important in the production of ATP with the aid of the ATP synthase contained in the inner membrane. The matrix contains a highly concentrated mixture of hundreds of enzymes, special mitochondrial [ribosomes](#), [tRNA](#), and several copies of the [mitochondrial DNA genome](#). Of the enzymes, the major functions include oxidation of [pyruvate](#) and [fatty acids](#), and the [citric acid cycle](#).<sup>[8]</sup>

Mitochondria have their own genetic material, and the machinery to manufacture their own [RNAs](#) and [proteins](#) (see: [protein biosynthesis](#)). A published human mitochondrial DNA sequence revealed 16,569 [base pairs](#) encoding 37 total genes: 22 [tRNA](#), 2 [rRNA](#), and 13 [peptide](#) genes.<sup>[24]</sup> The 13 mitochondrial [peptides](#) in humans are integrated into the inner mitochondrial membrane, along with [proteins](#) encoded by [genes](#) that reside in the host cell's [nucleus](#).

## Mitochondria-associated ER membrane (MAM)

The mitochondria-associated ER membrane (MAM) is another structural element that is increasingly recognized for its critical role in cellular physiology and [homeostasis](#). Once considered a technical snag in cell fractionation techniques, the alleged ER vesicle contaminants that invariably appeared in the mitochondrial fraction have been re-identified as membranous structures derived from the MAM—the interface between mitochondria and the ER.<sup>[25]</sup> Physical coupling between these two organelles had previously been observed in electron micrographs and has more recently been probed with [fluorescence microscopy](#).<sup>[25]</sup> Such studies estimate that at the MAM, which may comprise up to 20% of the mitochondrial outer membrane, the ER and mitochondria are separated by a mere 10–25 nm and held together by protein tethering complexes.<sup>[25][26][27]</sup>

Purified MAM from subcellular fractionation has shown to be enriched in enzymes involved in phospholipid exchange, in addition to channels associated with Ca<sup>2+</sup> signaling.<sup>[25][27]</sup> These hints of a prominent role for the MAM in the regulation of cellular

lipid stores and signal transduction have been borne out, with significant implications for mitochondrial-associated cellular phenomena, as discussed below. Not only has the MAM provided insight into the mechanistic basis underlying such physiological processes as intrinsic apoptosis and the propagation of calcium signaling, but it also favors a more refined view of the mitochondria. Though often seen as static, isolated 'powerhouses' hijacked for cellular metabolism through an ancient endosymbiotic event, the evolution of the MAM underscores the extent to which mitochondria have been integrated into overall cellular physiology, with intimate physical and functional coupling to the endomembrane system.

### *Phospholipid transfer*

The MAM is enriched in enzymes involved in lipid biosynthesis, such as phosphatidylserine synthase on the ER face and phosphatidylserine decarboxylase on the mitochondrial face.<sup>[28][29]</sup> Because mitochondria are dynamic organelles constantly undergoing [fission](#) and [fusion](#) events, they require a constant and well-regulated supply of phospholipids for membrane integrity.<sup>[30][31]</sup> But mitochondria are not only a destination for the phospholipids they finish synthesis of; rather, this organelle also plays a role in inter-organelle trafficking of the intermediates and products of phospholipid biosynthetic pathways, ceramide and cholesterol metabolism, and glycosphingolipid anabolism.<sup>[29][31]</sup>

Such trafficking capacity depends on the MAM, which has been shown to facilitate transfer of lipid intermediates between organelles.<sup>[28]</sup> In contrast to the standard vesicular mechanism of lipid transfer, evidence indicates that the physical proximity of the ER and mitochondrial membranes at the MAM allows for lipid flipping between opposed bilayers.<sup>[31]</sup> Despite this unusual and seemingly energetically unfavorable mechanism, such transport does not require ATP.<sup>[31]</sup> Instead, in yeast, it has been shown to be dependent on a multiprotein tethering structure termed the ER-mitochondria encounter structure, or ERMES, although it remains unclear whether this structure directly mediates lipid transfer or is required to keep the membranes in sufficiently close proximity to lower the energy barrier for lipid flipping.<sup>[31][32]</sup>

The MAM may also be part of the secretory pathway, in addition to its role in intracellular lipid trafficking. In particular, the MAM appears to be an intermediate destination between the rough ER and the Golgi in the pathway that leads to [very-low-density lipoprotein](#), or VLDL, assembly and secretion.<sup>[29][33]</sup> The MAM thus serves as a critical metabolic and trafficking hub in lipid metabolism.

### *Calcium signaling*

A critical role for the ER in calcium signaling was acknowledged before such a role for the mitochondria was widely accepted, in part because the low affinity of  $\text{Ca}^{2+}$  channels localized to the outer mitochondrial membrane seemed to fly in the face of this organelle's purported responsiveness to changes in intracellular  $\text{Ca}^{2+}$  flux.<sup>[25]</sup> But the presence of the MAM resolves this apparent contradiction: the close physical association between the two organelles results in  $\text{Ca}^{2+}$  microdomains at contact points that facilitate efficient  $\text{Ca}^{2+}$  transmission from the ER to the mitochondria.<sup>[25]</sup> Transmission occurs in response to so-called " $\text{Ca}^{2+}$  puffs" generated by spontaneous clustering and activation of [IP3R](#), a canonical ER membrane  $\text{Ca}^{2+}$  channel.<sup>[25][26]</sup>

The fate of these puffs—in particular, whether they remain restricted to isolated locales or integrated into  $\text{Ca}^{2+}$  waves for propagation throughout the cell—is determined in large part by MAM dynamics. Although reuptake of  $\text{Ca}^{2+}$  by the ER (concomitant with its release) modulates the intensity of the puffs, thus insulating mitochondria to a certain degree from high  $\text{Ca}^{2+}$  exposure, the MAM often serves as a firewall that essentially buffers  $\text{Ca}^{2+}$  puffs by acting as a sink into which free ions released into the cytosol can be funneled.<sup>[25][34][35]</sup> This  $\text{Ca}^{2+}$  tunneling occurs through the low-affinity  $\text{Ca}^{2+}$  receptor VDAC1, which recently has been shown to be physically [tethered](#) to the IP3R clusters on the ER membrane and enriched at the MAM.<sup>[25][26][36]</sup> The ability of mitochondria to serve as a  $\text{Ca}^{2+}$  sink is a result of the electrochemical gradient generated during oxidative phosphorylation, which makes tunneling of the cation an exergonic process.<sup>[36]</sup>

But transmission of  $\text{Ca}^{2+}$  is not unidirectional; rather, it is a two-way street. The properties of the  $\text{Ca}^{2+}$  pump SERCA and the channel IP3R present on the ER membrane facilitate feedback regulation coordinated by MAM function. In particular, clearance of  $\text{Ca}^{2+}$  by the MAM allows for spatio-temporal patterning of  $\text{Ca}^{2+}$  signaling because  $\text{Ca}^{2+}$  alters IP3R activity in a biphasic manner.<sup>[25]</sup> [SERCA](#) is likewise affected by mitochondrial feedback: uptake of  $\text{Ca}^{2+}$  by the MAM stimulates ATP production, thus providing energy that enables SERCA to reload the ER with  $\text{Ca}^{2+}$  for continued  $\text{Ca}^{2+}$  efflux at the MAM.<sup>[34][36]</sup> Thus, the MAM is not a passive buffer for  $\text{Ca}^{2+}$  puffs; rather it helps modulate further  $\text{Ca}^{2+}$  signaling through feedback loops that affect ER dynamics.

Regulating ER release of  $\text{Ca}^{2+}$  at the MAM is especially critical because only a certain window of  $\text{Ca}^{2+}$  uptake sustains the mitochondria, and consequently the cell, at homeostasis. Sufficient intraorganelle  $\text{Ca}^{2+}$  signaling is required to stimulate metabolism by activating dehydrogenase enzymes critical to flux through the citric acid cycle.<sup>[37]</sup> However, once  $\text{Ca}^{2+}$  signaling in the mitochondria passes a certain threshold, it stimulates the intrinsic pathway of apoptosis in part by collapsing the mitochondrial membrane potential required for metabolism.<sup>[25]</sup> Studies examining the role of pro- and anti-apoptotic factors support this model; for example, the anti-apoptotic factor Bcl-2 has been shown to interact with IP3Rs to reduce  $\text{Ca}^{2+}$  filling of the ER, leading to reduced efflux at the MAM and preventing collapse of the mitochondrial membrane potential post-apoptotic stimuli.<sup>[25]</sup> Given the need for such fine regulation of  $\text{Ca}^{2+}$  signaling, it is perhaps unsurprising that dysregulated mitochondrial  $\text{Ca}^{2+}$  has been implicated in several neurodegenerative diseases, while the catalogue of tumor suppressors includes a few that are enriched at the MAM.<sup>[36]</sup>

### *[Molecular basis for tethering](#)*

Recent advances in the identification of the [tethers](#) between the mitochondrial and ER membranes suggest that the scaffolding function of the molecular elements involved is secondary to other, non-structural functions. In yeast, ERMES, a multiprotein complex of interacting ER- and mitochondrial-resident membrane proteins, is required for lipid transfer at the MAM and exemplifies this principle. One of its components, for example, is also a constituent of the protein complex required for insertion of transmembrane beta-barrel proteins into the lipid bilayer.<sup>[31]</sup> However, a homologue of the ERMES complex has not been identified yet in mammalian cells. Other proteins implicated in scaffolding likewise have functions independent of structural tethering at the MAM; for example, ER-resident and mitochondrial-resident mitofusins form heterocomplexes that regulate the number of inter-organelle contact sites, although mitofusins were first

identified for their role in [fission](#) and [fusion](#) events between individual mitochondria.<sup>[25]</sup> Glucose-related protein 75 (grp75) is another dual-function protein. In addition to the matrix pool of grp75, a portion serves as a chaperone that physically links the mitochondrial and ER  $\text{Ca}^{2+}$  channels VDAC and IP3R for efficient  $\text{Ca}^{2+}$  transmission at the MAM.<sup>[25][26]</sup> Another potential tether is Sigma-1R, a non-opioid receptor whose stabilization of ER-resident IP3R may preserve communication at the MAM during the metabolic stress response.<sup>[38][39]</sup>

## **Perspective**

The MAM is a critical signaling, metabolic, and trafficking hub in the cell that allows for the integration of ER and mitochondrial physiology. Coupling between these organelles is not simply structural but functional as well and critical for overall cellular physiology and homeostasis. The MAM thus offers a perspective on mitochondria that diverges from the traditional view of this organelle as a static, isolated unit appropriated for its metabolic capacity by the cell. Instead, this mitochondrial-ER interface emphasizes the integration of the mitochondria, the product of an endosymbiotic event, into diverse cellular processes.

## **Organization and distribution**

Mitochondria are found in nearly all [eukaryotes](#).<sup>[40]</sup> They vary in number and location according to cell type. A single mitochondrion is often found in unicellular organisms. Conversely, numerous mitochondria are found in human liver cells, with about 1000–2000 mitochondria per cell, making up 1/5 of the cell volume.<sup>[8]</sup> The mitochondrial content of otherwise similar cells can vary substantially in size and membrane potential,<sup>[41]</sup> with differences arising from sources including uneven partitioning at cell divisions, leading to [extrinsic differences](#) in ATP levels and downstream cellular processes.<sup>[42]</sup> The mitochondria can be found nestled between [myofibrils](#) of [muscle](#) or wrapped around the [sperm flagellum](#).<sup>[8]</sup> Often they form a complex 3D branching network inside the cell with the [cytoskeleton](#). The association with the cytoskeleton determines mitochondrial shape, which can affect the function as well.<sup>[43]</sup> Mitochondria in cells are always distributed along microtubules and the distribution of these organelles is also correlated with the endoplasmic reticulum.<sup>[44]</sup> Recent evidence suggests that [vimentin](#), one of the components of the cytoskeleton, is also critical to the association with the cytoskeleton.<sup>[45]</sup>

## **Function**

The most prominent roles of mitochondria are to produce the energy currency of the cell, [ATP](#) (i.e., phosphorylation of [ADP](#)), through respiration, and to regulate cellular [metabolism](#).<sup>[9]</sup> The central set of reactions involved in ATP production are collectively known as the [citric acid cycle](#), or the [Krebs](#) cycle. However, the mitochondrion has many other functions in addition to the production of ATP.

## **Energy conversion**

A dominant role for the mitochondria is the production of [ATP](#), as reflected by the large number of proteins in the inner membrane for this task. This is done by oxidizing the

major products of [glucose](#): [pyruvate](#), and [NADH](#), which are produced in the cytosol.<sup>[9]</sup> This process of [cellular respiration](#), also known as [aerobic respiration](#), is dependent on the presence of [oxygen](#). When oxygen is limited, the glycolytic products will be metabolized by [anaerobic fermentation](#), a process that is independent of the mitochondria.<sup>[9]</sup> The production of ATP from glucose has an approximately 13-times higher yield during aerobic respiration compared to fermentation.<sup>[46]</sup> Recently it has been shown that plant mitochondria can produce a limited amount of ATP without oxygen by using the alternate substrate [nitrite](#).<sup>[47]</sup>

### *[Pyruvate and the citric acid cycle](#)*

Main articles: [Pyruvate dehydrogenase](#), [Pyruvate carboxylase](#) and [Citric acid cycle](#)

[Pyruvate](#) molecules produced by [glycolysis](#) are [actively transported](#) across the inner mitochondrial membrane, and into the matrix where they can be either [oxidized](#) and combined with [coenzyme A](#) to form CO<sub>2</sub>, [acetyl-CoA](#), and [NADH](#),<sup>[9]</sup> or they can be [carboxylated](#) (by [pyruvate carboxylase](#)) to form oxaloacetate. This latter reaction "fills up" the amount of oxaloacetate in the citric acid cycle (and is therefore an "anaplerotic reaction"), allowing the cycle to expand considerably when the tissue's energy needs (e.g. in [muscle](#)) are suddenly increased by activity.<sup>[48]</sup>

In the citric acid cycle all the intermediates (e.g. citrate, iso-citrate, alpha-ketoglutarate, succinate, fumarate, malate and oxaloacetate) are regenerated during each turn of the cycle. Adding more of any of these intermediates to the mitochondrion therefore means that that additional amount is retained in the cycle, increasing all the other intermediates as one is converted into the other. Hence the addition of any one of them to the cycle has an "anaplerotic" (filling up) effect, and its removal has a "cataplerotic" effect. These anaplerotic and cataplerotic reactions will, during the course of the cycle, increase or decrease the amount of oxaloacetate available to combine with acetyl-CoA to form citric acid. This in turn increases or decreases the rate of ATP production by the mitochondrion, and thus the availability of ATP to the cell.<sup>[48]</sup>

Acetyl-CoA, on the other hand, derived from pyruvate oxidation, or from the [beta-oxidation](#) of [fatty acids](#), is the only fuel to enter the citric acid cycle. With each turn of the cycle one molecule of acetyl-CoA is consumed for every molecule of oxaloacetate in the mitochondrial matrix, and is never regenerated. It is the oxidation of the acetate portion of acetyl-CoA that produces CO<sub>2</sub> and water, with the energy thus released captured in the form of ATP.<sup>[48]</sup>

In the liver, the carboxylation of [cytosolic](#) pyruvate into intra-mitochondrial oxaloacetate is an early step in the [gluconeogenic](#) pathway which converts [lactate](#) and deaminated [alanine](#) into glucose,<sup>[9][48]</sup> under the influence of high levels of [glucagon](#) and/or [epinephrine](#) in the blood.<sup>[48]</sup> Here the addition of oxaloacetate to the mitochondrion does not have a net anaplerotic effect, as another citric acid cycle intermediate (malate) is immediately removed from the mitochondrion to be converted into cytosolic oxaloacetate, which is ultimately converted into glucose, in a process that is almost the reverse of [glycolysis](#).<sup>[48]</sup>

The enzymes of the citric acid cycle are located in the mitochondrial matrix, with the exception of [succinate dehydrogenase](#), which is bound to the inner mitochondrial membrane as part of Complex II.<sup>[49]</sup> The citric acid cycle oxidizes the acetyl-CoA to



carbon dioxide, and, in the process, produces reduced cofactors (three molecules of [NADH](#) and one molecule of [FADH<sub>2</sub>](#)) that are a source of electrons for the [electron transport chain](#), and a molecule of [GTP](#) (that is readily converted to an ATP).<sup>[9]</sup>

### *NADH and FADH<sub>2</sub>: the electron transport chain*

The redox energy from NADH and FADH<sub>2</sub> is transferred to oxygen (O<sub>2</sub>) in several steps via the electron transport chain. These energy-rich molecules are produced within the matrix via the citric acid cycle but are also produced in the cytoplasm by [glycolysis](#). [Reducing equivalents](#) from the cytoplasm can be imported via the [malate-aspartate shuttle](#) system of [antiporter](#) proteins or feed into the electron transport chain using a [glycerol phosphate shuttle](#).<sup>[9]</sup> [Protein complexes](#) in the inner membrane ([NADH dehydrogenase \(ubiquinone\)](#), [cytochrome c reductase](#), and [cytochrome c oxidase](#)) perform the transfer and the incremental release of energy is used to pump [protons](#) (H<sup>+</sup>) into the intermembrane space. This process is efficient, but a small percentage of electrons may prematurely reduce oxygen, forming [reactive oxygen species](#) such as [superoxide](#).<sup>[9]</sup> This can cause [oxidative stress](#) in the mitochondria and may contribute to the decline in mitochondrial function associated with the aging process.<sup>[50]</sup>

As the proton concentration increases in the intermembrane space, a strong [electrochemical gradient](#) is established across the inner membrane. The protons can return to the matrix through the [ATP synthase](#) complex, and their potential energy is used to synthesize [ATP](#) from ADP and inorganic phosphate (P<sub>i</sub>).<sup>[9]</sup> This process is called [chemiosmosis](#), and was first described by [Peter Mitchell](#)<sup>[51][52]</sup> who was awarded the 1978 [Nobel Prize in Chemistry](#) for his work. Later, part of the 1997 Nobel Prize in Chemistry was awarded to [Paul D. Boyer](#) and [John E. Walker](#) for their clarification of the working mechanism of ATP synthase.<sup>[53]</sup>

### *Heat production*

Under certain conditions, protons can re-enter the mitochondrial matrix without contributing to ATP synthesis. This process is known as *proton leak* or *mitochondrial uncoupling* and is due to the [facilitated diffusion](#) of protons into the matrix. The process results in the unharnessed potential energy of the proton electrochemical gradient being released as heat.<sup>[9]</sup> The process is mediated by a proton channel called [thermogenin](#), or [UCP1](#).<sup>[54]</sup> Thermogenin is a 33 kDa protein first discovered in 1973.<sup>[55]</sup> Thermogenin is primarily found in [brown adipose tissue](#), or brown fat, and is responsible for non-shivering thermogenesis. Brown adipose tissue is found in mammals, and is at its highest levels in early life and in hibernating animals. In humans, brown adipose tissue is present at birth and decreases with age.<sup>[54]</sup>

## **The prolactin responses to active and passive heating in man**

Low, D, Purvis, A, Reilly, et al (*Exper. Physiology* 90.6, pp 909-917 (2005))

The aim of this study was to compare the prolactin and blood pressure responses at identical core temperatures during active and passive heat stresses, using prolactin as an indirect marker of central fatigue. Twelve male subjects cycled to exhaustion at 60% maximal oxygen uptake ( $\dot{V}_{O_{2peak}}$ ) in a room maintained at 33°C (active). In a second

trial they were passively heated (passive) in a water bath ( $41.56 \pm 1.65^{\circ}\text{C}$ ) until core temperature was equal to the core temperature observed at exhaustion during the active trial. Blood samples were taken from an indwelling venous cannula for the determination of serum prolactin during active heating and at corresponding core temperatures during passive heating. Core temperature was not significantly different between the two methods of heating and averaged  $38.81 \pm 0.53$  and  $38.82 \pm 0.70^{\circ}\text{C}$  (data expressed as means  $\pm$  s.d.) at exhaustion during active heating and at the end of passive heating, respectively ( $P > 0.05$ ). Mean arterial blood pressure was significantly lower throughout passive heating (active,  $73 \pm 9$  mmHg; passive,  $62 \pm 12$  mmHg;  $P < 0.01$ ). Despite the significantly reduced blood pressure responses during passive heating, during both forms of heating the prolactin response was the same (active,  $14.9 \pm 12.6$  ng ml $^{-1}$ ; passive,  $13.3 \pm 9.6$  ng ml $^{-1}$ ; n.s.). These results suggest that thermoregulatory, i.e. core temperature, and not cardiovascular afferents provide the key stimulus for the release of prolactin, an indirect marker of central fatigue, during exercise in the heat.

The capacity for prolonged exercise is diminished in hot environments relative to normothermic conditions ([Galloway & Maughan, 1997](#); [Parkin et al. 1999](#)). The precise mechanism(s) for this decreased endurance capacity during exercise in the heat is unknown. However, possible causes include an intolerable thermoregulatory strain, cerebral perturbations, central nervous system disturbances, high cardiovascular strain and altered skeletal muscle function ([Febbraio, 2000](#); [Cheung & Sleivert, 2004](#)). Support for the proposal that an intolerable thermoregulatory strain mediates fatigue during prolonged exercise in the heat comes from studies in which subjects reached the point of voluntary fatigue at similar core temperatures despite various manipulations to alter the baseline core temperature and/or the rate of increase in core temperature ([Nielsen et al. 1993](#); [Cheung & McLellan, 1998](#); [Gonzalez-Alonso et al. 1999](#); [Gregson et al. 2002](#)). It is thought that the fatigue that ensues at an intolerable core temperature is mediated by a reflex inhibition within the central nervous system, characterized by an increased reluctance of subjects to continue exercise, which acts as a safety brake to stop exercise and thus prevent any further increases in core and brain temperature ([Bruck & Olschewski, 1987](#); [Nielsen & Nybo, 2003](#)). Furthermore, it has also been proposed that this reflex inhibition possibly occurs via alterations in central serotonergic and dopaminergic activity ([Pitsiladis et al. 2002](#); [Bridge et al. 2003](#)).

Passive and active (exercise-induced) elevations in core temperature increase central serotonergic activity ([Hori & Harada, 1976](#); [Bridge et al. 2003](#)). The central serotonergic and dopaminergic transmitter systems have been implicated in the thermoregulatory control of body temperature and are thought to mediate thermoregulatory heat loss responses such as vasodilatation ([Cox et al. 1980](#); [Lee et al. 1985](#)). In addition, alterations in the activity of the central serotonergic and dopaminergic systems can also result in changes in behaviour, including arousal and motor control, that could reduce the motivation to continue exercise, which is evident at fatigue during exercise in hot conditions ([Young, 1991](#); [Jacobs & Fornal, 1993](#); [Nielsen et al. 2001](#)). However, the measurement of central serotonergic and dopaminergic activity has practical limitations in humans, so the anterior pituitary hormone prolactin is often used as an indirect marker of central serotonergic and dopaminergic activity, since its release is regulated by the central serotonergic and dopaminergic systems ([Freeman et al. 2000](#); [Bridge et al. 2003](#)). Serotonergic neurones in the dorsal raphe nucleus, located in the brainstem, stimulate the secretion of prolactin from serotonergic nerve terminals in the

hypothalamus through activation of serotonergic receptors ([Van de Kar et al. 1996](#)). Hypothalamic dopaminergic neurones that secrete dopamine into the pituitary portal vessels also tonically inhibit the secretion of prolactin ([Van de Kar et al. 1996](#)). Significant increases in prolactin (which peak at the point of exhaustion) are evident during exercise in the heat that leads to an intolerable thermoregulatory strain ([Brisson et al. 1989](#); [Pitsiladis et al. 2002](#)). This suggests alterations in central serotonergic and dopaminergic activity, in response to the increased core temperature, that could contribute to the reduced motivation to continue exercise, and subsequently fatigue, in the heat. The prolactin responses at exhaustion were also significantly related to the core temperature responses at exhaustion in these studies, further supporting a link between thermal limits of tolerance and the occurrence of this proposal of central fatigue.

A high ambient temperature increases the cardiovascular strain during exercise, because of the concurrent demands of the active musculature and the cutaneous circulation for blood flow ([Gonzalez-Alonso et al. 1995, 1997](#)). A high cardiovascular strain has also been proposed as a possible mediator of fatigue during prolonged exercise in hot conditions ([Cheung & Sleivert, 2004](#)). Despite this, the relationship between cardiovascular strain and fatigue during prolonged exercise in hot conditions has not been completely researched. The competition for blood flow between the cutaneous circulation, for heat dissipation, and the active musculature, for substrate delivery and removal of metabolic byproducts, during prolonged exercise in hot conditions threatens the effective regulation of blood pressure ([Rowell, 1993](#)). Should blood pressure fall to deleterious levels, cerebral blood flow will decrease and cause syncope (fainting). Reductions in mean arterial blood pressure ([Gonzalez-Alonso et al. 1997](#)) and cerebral blood flow ([Nybo & Nielsen, 2001b](#)) have been reported during prolonged exercise in hot conditions. The control of blood pressure has also been linked with changes in serotonergic brain activity in the rodent model ([Pergola & Alper, 1991, 1992](#)). Marked competition for blood flow between the cutaneous circulation and the active musculature during exercise under hot conditions may result in a reduction of blood pressure to potentially dangerously low levels, and low blood pressure may provide an additional signal, alongside or even instead of core temperature, to induce central fatigue, stop exercise and therefore maintain blood pressure and cerebral blood flow.

Separating the core temperature and blood pressure responses during exercise in hot conditions is difficult to achieve. Active (exercise) and passive methods of heat stress invoke similar thermoregulatory but different cardiovascular responses ([Powers et al. 1982](#)). The different cardiovascular responses to active and passive methods of heating with similar rises in core temperature relate to the exercise-induced demand for active skeletal muscle blood flow during active heating. Subsequently, attenuated increases in adrenaline, noradrenaline, heart rate, cardiac output and a lower blood pressure are evident during passive heating ([Rowell et al. 1970](#); [Powers et al. 1982](#); [Minson et al. 1998](#)). In addition, during passive heating the core temperature threshold for increases in skin blood flow is lower, and increases in skin blood flow are not attenuated at core temperatures of  $\sim 38.0^{\circ}\text{C}$ , as they are during exercise, which also contributes to a lower blood pressure during passive heating ([Kenney & Johnson, 1992](#)). A direct comparison of an active and passive heating challenge and their thermoregulatory, cardiovascular and prolactin responses will therefore permit better insight into the mechanisms underlying changes in prolactin activity, and possibly central serotonergic and



dopaminergic activity, relating to central fatigue during exercise under hot conditions. One previous study has examined the prolactin responses to passive and active (bicycle exercise) heating and reported greater increases in prolactin during passive heating ([Brisson et al. 1991](#)). In that study, however, no cardiovascular variables were measured, and the subjects were heated for set periods of time and not in relation to target increases in core temperature. Therefore, the aim of this study was to compare the prolactin and blood pressure responses at identical core temperatures during active and passive heat stresses. It is hypothesized that, in comparison to active heating, there will be a greater prolactin response alongside the lower blood pressure response during passive heating, suggesting that peripheral blood flow displacement and an attendant drop in arterial blood pressure stimulates the release of prolactin during exercise in hot conditions.

## Methods

### *Subjects and experimental design*

After local ethical committee approval, 12 active male subjects gave their written informed consent to participate in this study. Their physical characteristics (means  $\pm$  1 s.d.) were: age,  $23.9 \pm 3.7$  years; height,  $177.8 \pm 4.8$  cm; body mass,  $75.7 \pm 4.4$  kg; maximal oxygen uptake ( $\dot{V}_{O_{2peak}}$ ),  $50.0 \pm 6.4$  ml kg<sup>-1</sup> min<sup>-1</sup>. For the active heating trial, subjects cycled to exhaustion at 60%  $\dot{V}_{O_{2peak}}$  at 70–80 cycles min<sup>-1</sup> on a semirecumbent cycle ergometer (Kettler Sport, Redditch, Worcestershire, UK) in a room maintained at 33°C and constant humidity (40%). They then conducted (at least 7 days later) a passive heating trial, immersed in a water bath up to the xiphoid process with the arms out until they reached the core temperature at exhaustion in the active heating trial. All procedures were performed according to the Declaration of Helsinki.

### *Pre-trial phase*

Subjects reported to the laboratory and were required to insert a rectal probe (ELLAB, Kings Lynn, Norfolk, UK) 10 cm beyond the anal sphincter. Skin thermistors (ELLAB) were attached to four sites on the right-hand side of the body (anterior thigh, medial calf, chest and anterior forearm) for the determination of weighted mean skin temperature according to [Ramanathan \(1964\)](#). Two laser Doppler flowmetry skin probes (Perimed, Bury St Edmunds, Suffolk, UK) were attached to the anterior side of a forearm for the measurement of forearm skin blood flow. Subjects were also fitted with a heart rate monitor (Polar, Kempele, Finland) for the determination of heart rate using short-range telemetry.

### *Heating phase*

During both active and passive heating trials, heart rate (Polar Accurex Plus, Kempele, Finland), core and skin temperature (ELLAB) were monitored continuously and sampled each minute. Forearm skin blood flow (in volts; Periflux system 5000, Perimed, Jarfalla, Sweden) and blood pressure (in mmHg; (PortaPress Model 2, TPD Biomedical Instrumentation, Amsterdam, The Netherlands) from two digits of the same forearm on which skin blood flow was being measured were also monitored continuously throughout both heating trials and sampled every 20 s. The arm from which skin blood

flow and blood pressure were measured was placed at heart level throughout each trial. Forearm cutaneous vascular conductance (CVC) was used as an index of skin blood flow and was calculated by the ratio of forearm skin blood flow to blood pressure (volts mmHg<sup>-1</sup>). Subjects also provided ratings of thermal discomfort ([Toner et al. 1986](#)) during both trials. Data were recorded at 20 min intervals and at exhaustion during the active heating trial. During the passive heating trial, data were recorded at the corresponding core temperatures that were recorded at the 20 min intervals and at exhaustion during the active heating trial. Subjects were passively heated until they reached the same core temperature at exhaustion as in the active heating trial.

### **Prolactin analysis**

An indwelling venous cannula (Becton-Dickinson, Oxford, UK) was inserted in a vein in the antecubital crease or anterior forearm opposite to the forearm where skin blood flow was measured. The cannula was kept patent with 5–10 ml of isotonic saline (Becton-Dickinson) after insertion and after each blood sample. Blood samples were drawn pre-trial, at 20 min intervals and at exhaustion during the active exercise trial. During the passive heating trial, samples were drawn at the corresponding core temperatures recorded at the 20 min intervals and at exhaustion during the active heating trial. Samples were dispensed into serum separation tubes (HMS, Northampton, UK) and were left at room temperature for 1 h before being centrifuged, with the supernatant subsequently being frozen (–20.0°C) for analysis at a later stage. Duplicate haematocrit (Micro haematocrit tubes, L.I.P. Equipment, Bradford, Yorkshire, UK) and haemoglobin samples (Microcuvettes, Hemocue, Angelholm, Sweden) were also collected to calculate changes in plasma volume relative to the baseline sample according to [Dill & Costill \(1974\)](#). Serum prolactin was determined using an enzymeimmunoassay technique (DRG Instruments GmbH, Marburg, Germany) on a fully automated immunoassay system (Triturus, Grifols, Cambridge, UK). All samples were analysed in duplicate in one batch and corrected for changes in plasma volume.

### **Statistics**

Data for all subjects were averaged and are expressed as means  $\pm$  1 s.d. Statistical analysis of the thermoregulatory, cardiovascular and prolactin responses at the same core temperatures was carried out using a two-factor (core temperature and condition) repeated measures ANOVA. When significant main effects of each factor(s) and/or an interaction of the two factors were found, multiple comparisons within and between each factor(s) were made according to [Atkinson \(2002\)](#). When the assumption of sphericity was violated, ANOVA results were adjusted using Greenhouse Geisser or Huynh-Feldt values according to [Atkinson \(2002\)](#). An alpha level of 0.05 was taken to indicate statistical significance.

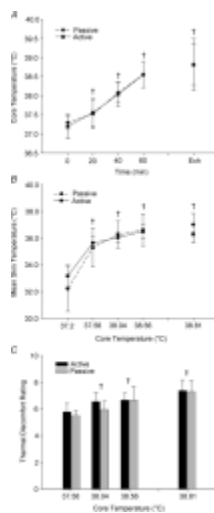
### **Results**

The average temperature of the water bath during the passive heating trial was  $41.56 \pm 1.65^\circ\text{C}$ . The durations of the active and passive exposures were not different ( $T_{11} = 0.45$ , n.s.), averaging  $63.67 \pm 11.90$  and  $59.50 \pm 32.60$  min for the active and passive heating trials, respectively. Due to technical difficulties with the water bath, two of the 12 subjects could not reach their core temperature attained at exhaustion in the active

heating trial and their passive heating trials were terminated before this. The data from these two subject's trials were therefore omitted from the statistical analysis.

### Thermoregulatory responses

Core temperature increased significantly and in a similar fashion across both trials ( $F_{1,36,10.88} = 44.87$ ,  $P < 0.01$ ; see [Fig. 1A](#)). There was no difference in the core temperatures attained at any time point between protocols ( $F_{1,8} = 0.73$ , n.s.). Core temperature averaged  $38.81 \pm 0.53$  and  $38.82 \pm 0.70^\circ\text{C}$  (range  $38.2\text{--}39.7^\circ\text{C}$ ) at exhaustion during the active heating trial and at the end of the passive heating trial, respectively. Therefore, the remainder of the data were expressed in relation to the core temperatures at the sampling points during the active heating trial and not in relation to time.



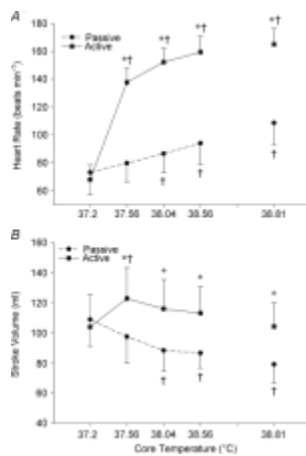
**Figure 1. Average core (A) and mean skin temperature responses (B) and thermal discomfort ratings (C) during active and passive methods of heating † $P < 0.05$  versus baseline;  $n = 11$  for core temperature,  $n = 6$  for mean skin temperature and  $n = 11$  for thermal discomfort rating results. Exh, exhaustion.**

Owing to technical errors with skin temperature measurement during four of the passive heating trials, statistical analysis on the mean skin temperature data was carried out on six subjects. Mean skin temperature increased significantly over time in both the active and passive heating trials ( $F_{1,28,6.24} = 58.41$ ,  $P < 0.01$ ; see [Fig. 1B](#)), but was not significantly different between the protocols ( $F_{1,5} = 0.45$ , n.s.), averaging  $35.77 \pm 0.76$  and  $37.03 \pm 0.83^\circ\text{C}$  at exhaustion during the active and at the end of the passive heating trials, respectively. Ratings of thermal discomfort significantly increased with core temperature ( $F_{1,3,10.40} = 29.91$ ,  $P < 0.01$ ; see [Fig. 1C](#)) and were not different between the two methods of heating ( $F_{1,8} = 4.58$ , n.s.). The average ratings of thermal discomfort at exhaustion during the active and at the end of the passive heating trials were  $7.25 \pm 1.00$  and  $7.30 \pm 1.00$ , respectively, with a rating of 7 representing 'very hot'.

### Cardiovascular responses

The heart rate and stroke volume responses to active and passive heating are displayed in [Fig. 2](#). Heart rate increased significantly with core temperature in both

forms of heating ( $F_{2.56,12.79} = 111.56$ ,  $P < 0.01$ ; see [Fig. 2A](#)). However, the increase in heart rate was significantly greater during the active heating trial ( $F_{2.50,12.52} = 51.44$ ,  $P < 0.01$ ). Heart rate averaged  $109 \pm 16$  and  $167 \pm 14$  beats  $\text{min}^{-1}$  at the end of the passive heating trial and at exhaustion during the active heating trial, respectively ( $P < 0.01$ ). As a result of technical errors with the blood pressure measurements during two subjects' passive heating trials, statistical analyses of the cardiovascular data were carried out on nine subjects. Both passive and active heating caused stroke volume to decrease significantly with increasing core temperature ( $F_{2.70,18.88} = 8.37$ ,  $P < 0.01$ ; see [Fig. 2B](#)). During the active heating trial, after an initial increase at 20 min, stroke volume decreased towards resting baseline levels at exhaustion ( $F_{3,21} = 20.68$ ,  $P < 0.01$ ). During the course of passive heat exposure, stroke volume decreased with progressive increases in core temperature ( $F_{1,7} = 53.38$ ,  $P < 0.01$ ). Stroke volume averaged  $79 \pm 12$  and  $105 \pm 13$  ml at the end of the passive heating trial and at exhaustion during the active heating trial, respectively ( $P < 0.01$ ).



**Figure 2. Average heart rate (A) and stroke volume responses (B) during active and passive methods of heating** † $P < 0.05$  versus baseline; \* $P < 0.01$ , Active versus Passive;  $n = 11$  for heart rate and  $n = 9$  for stroke volume.

Cardiac output increased significantly during both forms of heating ( $F_{2.88,23.07} = 184.95$ ,  $P < 0.01$ ; see [Fig. 3A](#)) but the increase in cardiac output was significantly higher during active than during passive heating ( $F_{2.06,16.47} = 63.89$ ,  $P < 0.01$ ). The average values of cardiac output at exhaustion during the active and at the end of the passive heating trials were  $16.9 \pm 2.1$  and  $8.3 \pm 1.7$   $\text{l min}^{-1}$ , respectively ( $P < 0.01$ ). Mean arterial pressure was significantly lower during passive heating ( $F_{1,8} = 28.94$ ,  $P < 0.01$ ; see [Fig. 3B](#)). During the active heat stress, mean arterial pressure was maintained relative to baseline but during passive heating, mean arterial pressure was significantly decreased below baseline levels ( $F_{1.80,14.37} = 12.24$ ,  $P < 0.01$ ), averaging  $60 \pm 9$  and  $75 \pm 8$  mmHg for the passive and active heating trials, respectively ( $P < 0.01$ ).

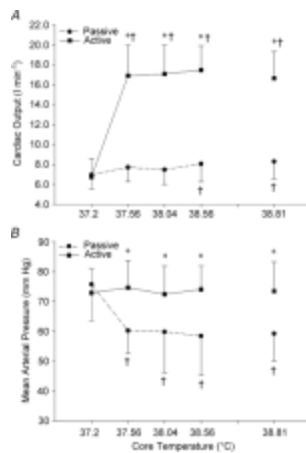


Figure 3. **Average cardiac output (A) and mean arterial pressure responses (B) during active and passive methods of heating** † $P < 0.05$  versus baseline; \* $P < 0.01$ , Active versus Passive;  $n = 9$  for cardiac output and mean arterial pressure.

Forearm skin blood flow increased significantly with core temperature in both forms of heating ( $F_{2,8} = 2.47$ ,  $P < 0.05$ ; see Fig. 4). During the passive heat stress, skin blood flow was significantly elevated above that during the active heat stress at 38.08°C until the end of the passive exposure ( $F_{1,8} = 4.94$ ,  $P < 0.05$ ). At exhaustion during active heating and at the same core temperature during passive heating, forearm CVC increased to  $314.82 \pm 91.62$  and  $444.87 \pm 170.75\%$  from baseline, respectively ( $P < 0.05$ ).

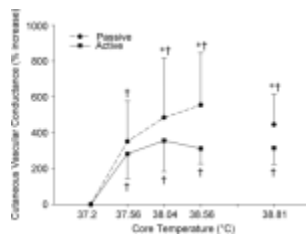


Figure 4. **Average forearm skin blood flow response during active and passive methods of heating** † $P < 0.05$  versus baseline, \* $P < 0.05$ , Active versus Passive.

### Prolactin responses

As a result of errors with blood sampling during two subjects' passive heating trials, statistical analysis of the prolactin data was carried out on nine subjects. Serum prolactin increased significantly during both active and passive heating ( $F_{1,05,7.36} = 4.44$ ,  $P < 0.05$ ; see Fig. 5). There was no difference in the prolactin response between the methods of heating ( $F_{1,8} = 0.10$ , n.s.). The average serum prolactin values at exhaustion during active heating and at the end of the passive heating trial were  $26.0 \pm 20.3$  and  $23.1 \pm 16.6$  ng ml<sup>-1</sup> (range 8–85 ng ml<sup>-1</sup>), respectively. The prolactin and the core temperature responses at the end of both forms of heating were significantly correlated ( $r^2 = 0.870$ ,  $P < 0.0001$ ,  $n = 22$ ).

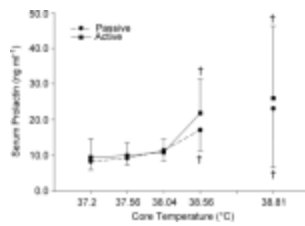


Figure 5. **Serum prolactin responses during active and passive methods of heating** † $P < 0.05$  versus baseline,  $n = 9$ .

## Discussion

The aim of this investigation was to compare the prolactin and the cardiovascular responses at identical core temperatures during an active (exercise) heating trial and a passive (water immersion) heating trial. The passive heating trial was used in this study to obtain similar core temperatures to those achieved in an active (exercise) heat stress but with different cardiovascular responses owing to the absence of exercise. This design provided an insight into the mechanisms of prolactin release and an indication of central serotonergic and dopaminergic activity relating to central fatigue during exercise-induced hyperthermia. The specific hypothesis tested was that peripheral blood flow displacement, and an attendant drop in arterial blood pressure and therefore cerebral blood flow, stimulates the release of prolactin during exercise in hot conditions.

Core and mean skin temperatures and thermal discomfort ratings were not different between the two methods of heating. Increases in heart rate and cardiac output in response to increases in core temperature were significantly attenuated during passive heating. Alongside these similar thermoregulatory responses to passive and active heating, during passive heating skin blood flow was significantly increased and stroke volume and mean arterial blood pressure were significantly reduced. These responses are consistent with previous research that has investigated the cardiovascular responses to active and passive heat stresses ([Rowell et al. 1970](#); [Powers et al. 1982](#); [Minson et al. 1998](#)). Despite these altered cardiovascular responses to passive, relative to active, heating the prolactin responses were the same during both methods of heating. The core temperature and prolactin responses at the end of both forms of heating were also significantly correlated ( $r^2 = 0.870$ ,  $P < 0.0001$ ,  $n = 22$ ). These results suggest that thermoregulatory afferents, i.e. increases in core temperature, are probably the key stimulus for prolactin release, which is in agreement with previous work ([Brisson et al. 1989](#); [Bridge et al. 2003](#)).

The secretion of the anterior pituitary hormone prolactin is regulated by the central serotonergic and dopaminergic systems, and prolactin is therefore often used as an indirect marker of central serotonergic and dopaminergic activity ([Freeman et al. 2000](#); [Bridge et al. 2003](#)). Central serotonergic and dopaminergic activity has been shown to increase during passive and active (exercise-induced) elevations in core temperature ([Hori & Harada, 1976](#); [Bridge et al. 2003](#)), and alterations in the activity of both of these systems have been implicated in the thermoregulatory control of body temperature, by mediating vasodilatation ([Cox et al. 1980](#); [Lee et al. 1985](#)). In addition, central serotonergic and dopaminergic pathways have also been shown to modulate changes in behavioural states, such as arousal and motor control ([Young, 1991](#); [Jacobs & Fornal, 1993](#)). In the present study, prolactin significantly increased during both forms of



heating and peaked at exhaustion during active heating, consistent with previous data ([Pitsiladis et al. 2002](#); [Bridge et al. 2003](#)), suggesting that alterations in the activity of the central serotonergic and dopaminergic systems occur at the same time as when core temperature and thermal discomfort have increased to intolerable levels and when the desire to continue exercise is reduced and fatigue ensues.

A number of mechanisms for a decreased endurance capacity during exercise in the heat have been proposed, including an intolerable thermoregulatory strain, cerebral perturbations, central nervous system disturbances, high cardiovascular strain and altered skeletal muscle function ([Febbraio, 2000](#); [Cheung & Sleivert, 2004](#)). Despite an alteration in the initial baseline core temperature or the rate of increase in core temperature during exercise in a hot environment using precooling and prewarming manoeuvres and conducting heat acclimation programmes, studies have shown that fatigue during prolonged exercise in the heat occurs at a similar intolerable thermoregulatory strain ([Nielsen et al. 1993](#); [Cheung & McLellan, 1998](#); [Gonzalez-Alonso et al. 1999](#); [Gregson et al. 2002](#)). Increases in core temperature to intolerable levels at exhaustion during exercise in hot conditions occur at the same time as maximal or near-maximal ratings of perceived exertion ([Nybo & Nielsen, 2001 c](#)), a significantly decreased level of arousal ([Nielsen et al. 2001](#)) and a significant reduction of the voluntary activation of a previously exercised muscle ([Nybo & Nielsen, 2001 a](#)). Other experimental work has shown that increases in prolactin at exhaustion are significantly related to core temperature and ratings of perceived exertion at exhaustion during prolonged exercise in hot conditions ([Pitsiladis et al. 2002](#); [Bridge et al. 2003](#); [Low et al. 2005](#)). Collectively, these results support a possible link between alterations in central serotonergic and dopaminergic activity and the attainment of thermal limits of tolerance during prolonged exercise in hot conditions, which could lead to a reduced motivation to continue exercise arising from central command in order to protect against any further potentially damaging increases in core and brain temperature ([Nielsen & Nybo, 2003](#)).

Exercise in a hot environment that causes thermoregulatory strain is also accompanied by an increased cardiovascular strain ([Rowell, 1993](#)). Decreases in cardiac output and stroke volume alongside decreases in mean arterial pressure and cerebral blood flow are evident during exercise in the heat ([Gonzalez-Alonso et al. 1995, 1997](#); [Nybo & Nielsen, 2001 b](#)). Should blood pressure and subsequently brain blood flow fall to dangerously low levels, syncope can occur. Therefore, decreases in blood pressure and cerebral blood flow to syncopal levels could provide afferent feedback to the brain to instigate central fatigue in order to terminate exercise. In the rodent model, intercerebroventricular injections of serotonin increase blood pressure ([Pergola & Alper, 1991, 1992](#)), suggesting a link between the control of blood pressure and serotonergic brain activity, one of the neurotransmitter systems that has been associated with the onset of central fatigue during exercise in hot conditions. In the present study, active and passive methods of heating invoked similar increases in core temperature to intolerable levels but a significantly lower blood pressure response occurred during passive heating. Despite these differences in the blood pressure response, the prolactin increases were the same during these two forms of heating, which indicates that a high cardiovascular strain and a reduced blood pressure do not contribute to changes in prolactin release and possibly central serotonergic and dopaminergic activity. In support of these findings, baroreflex control of blood pressure is not inhibited during exercise ([Mack et al. 1988](#); [Potts et al. 1993](#)) or during hyperthermia *per se* ([Crandall et al. 1999](#);

[Crandall, 2000](#)), indicating that blood pressure is effectively regulated during exercise in hot conditions. In addition, the decrease in cerebral blood flow occurring during exercise in hot conditions that leads to an intolerable core temperature ([Nybo & Nielsen, 2001b](#)) does not reach a level low enough to cause syncope ([Van Lieshout et al. 2003](#)). However, it is important that in the present study and others that have investigated mechanisms of fatigue during prolonged exercise in the heat, owing to methodological considerations, bicycle ergometers rather than upright running postures were used as the exercise mode. An upright running posture compared to a seated bicycle posture will probably invoke a greater orthostatic stress during exercise ([Rowell, 1993](#)) and therefore the (in)effective control of blood pressure may possibly play a more important role in fatigue during prolonged exercise in the heat during running. This warrants further investigation.

In a previous study that examined the prolactin responses to 30 min of passive heating and 45 min of activity (bicycle exercise at 65%  $\dot{V}_{O_{2peak}}$  in a 41°C environment) [Brisson et al. \(1991\)](#) reported larger increases in prolactin (although no statistical analysis was performed) during passive heating, despite similar increases in rectal temperature (1.6°C). The intensity of exercise, the temperature of the water in which subjects were immersed and the increases in rectal temperature in the study of [Brisson et al. \(1991\)](#) are similar to those in the present study, but the reasons for larger prolactin increases during passive heating in the study of [Brisson et al. \(1991\)](#) compared with the present study are not clear. The prolactin response to exercise in the heat is significantly reduced with facial fanning that reduces mean overall skin temperature but does not change core (rectal) temperature ([Brisson et al. 1991](#); [Armada da Silva et al. 2004](#)). In the study of [Brisson et al. \(1991\)](#), subjects were immersed up to the chin, in contrast to the sternum with arms out in the present study, potentially resulting in a greater mean skin temperature and therefore greater prolactin response during the passive, relative to the active, heat stress in the study of [Brisson et al. \(1991\)](#). Another possible reason for the discrepancy is the difference in the components of blood analysed for prolactin in the present study and the study of [Brisson et al. \(1991\)](#); serum *versus* plasma, respectively).

It has been shown in an animal model that increases in prolactin can be mediated by stress that is induced by exposing the animals to ether or by restraining them ([Freeman et al. 2000](#)). Although not performed in this study, to serve as a control condition in the study of [Brisson et al. \(1989\)](#) subjects sat and rested on the cycle ergometer for the same amount of time and under the same conditions as in the active exercise trial with no change in core temperature. In addition, subjects were also immersed in the water bath at a thermoneutral temperature for the same duration as the passive heat stress and no change in core temperature was evident. In both of these control trials no change in prolactin was observed, indicating that being required to be seated on a cycle ergometer or in a water bath, as in the present study, do not *per se* cause changes in prolactin. In addition, the thermal discomfort ratings at the end of both forms of heating in the present study indicated that the subjects terminated their trials at the point when thermal discomfort was almost maximal.

In conclusion, active (exercise-induced) and passive hyperthermia that invoked identical core temperatures yielded similar prolactin responses. This was despite different cardiovascular responses to the two forms of body heating. These results suggest that increases in core temperature, not alterations in peripheral blood flow and blood



pressure, provide the key stimulus for prolactin release, which may be a marker of central serotonergic and dopaminergic activity relating to central fatigue during exercise in hot conditions.

## Prolactin

**Prolactin (PRL)**, also known as luteotropic hormone or luteotropin, is a protein also known as a **peptide hormone** and is encoded by the PRL gene. Prolactin is best known in humans for its role in enabling [female mammals to produce milk](#), but it is influential over a vast number of important functions (over 300 separate actions of PRL have been reported in various vertebrates. Prolactin is secreted from the [pituitary gland](#) in response to eating, mating, estrogen treatment, ovulation, and nursing. Prolactin is secreted in a pulsatile fashion in between these events. Prolactin also plays an essential role in metabolism, regulation of the immune system, and pancreatic development.

Prolactin also acts in a [cytokine](#)-like manner and as an important regulator of the [immune system](#). It has important cell cycle related functions as a growth-, differentiating- and anti-[apoptotic](#) factor. As a growth factor, binding to cytokine like receptors, it also has profound influence on [hematopoiesis](#), [angiogenesis](#) and is involved in the regulation of blood clotting through several pathways. The hormone acts in endocrine, autocrine, and paracrine manner through the [prolactin receptor](#) and a large number of [cytokine receptors](#).<sup>[1]</sup>

Pituitary prolactin secretion is regulated by [endocrine](#) neurons in the [hypothalamus](#), the most important ones being the neurosecretory [tuberoinfundibulum](#) (TIDA) neurons of the [arcuate nucleus](#), which secrete [dopamine](#) (aka Prolactin Inhibitory Hormone) to act on the [D<sub>2</sub> receptors](#) of [lactotrophs](#), causing [inhibition](#) of prolactin secretion. [Thyrotropin-releasing factor](#) (thyrotropin-releasing hormone) has a [stimulatory](#) effect on prolactin release, however Prl is the only [adenohypophyseal](#) hormone whose principal control is inhibitory.

## Effects

Prolactin has a wide range of effects. It stimulates the [mammary glands](#) to produce milk ([lactation](#)): increased serum concentrations of prolactin during [pregnancy](#) cause enlargement of the [mammary glands](#) of the breasts and prepare for the production of milk. Prolactin provides

the body with [sexual gratification](#) after [sexual acts](#): The [hormone](#) counteracts the effect of [dopamine](#), which is responsible for [sexual arousal](#). This is thought to cause the [sexual refractory period](#). The amount of prolactin can be an indicator for the amount of sexual satisfaction and relaxation. Unusually high amounts are suspected to be responsible for [impotence](#) and loss of [libido](#).

Highly elevated levels of prolactin decrease the levels of sex hormones — [estrogen](#) in women and [testosterone](#) in men.<sup>[6]</sup> The effects of mildly elevated levels of prolactin are much more variable, in women both substantial increase or decrease of estrogen levels may result.

Prolactin is sometimes classified as a [gonadotropin](#)<sup>[7]</sup> although in humans it has only a weak luteotropic effect while the effect of suppressing classical gonadotropic hormones is more important.<sup>[8]</sup> Prolactin within the normal reference ranges can act as a weak gonadotropin but at the same time suppresses GnRH secretion. The exact mechanism by which it inhibits GnRH is poorly understood although expression of prolactin receptors (PRL-R) have been demonstrated in rat's hypothalamus, the same has not been observed in GnRH neurons.<sup>[9]</sup> Physiologic levels of prolactin in males enhance [luteinizing hormone](#)-receptors in [Leydig cells](#), resulting in testosterone secretion, which leads to spermatogenesis.<sup>[10]</sup>

Prolactin also stimulates proliferation of [oligodendrocyte precursor cells](#). These cells differentiate into [oligodendrocytes](#), the cells responsible for the formation of [myelin](#) coatings on [axons](#) in the [central nervous system](#).<sup>[11]</sup> Prolactin also has a number of other effects including contributing to [pulmonary surfactant](#) synthesis of the fetal [lungs](#) at the end of the pregnancy and [immune tolerance](#) of the fetus by the maternal organism during pregnancy.

Prolactin promotes [neurogenesis](#) in maternal and [fetal](#) brains.<sup>[13][14]</sup>

Published studies have shown that prolactin can reduce the motivation to continue exercise, which is evident at fatigue during exercise in hot conditions. However, the measurement of central serotonergic and dopaminergic activity has practical limitations in humans, so the

anterior pituitary hormone prolactin is often used as an indirect marker of central serotonergic and dopaminergic activity, since its release is regulated by the central serotonergic and dopaminergic systems. Serotonergic neurones in the dorsal raphe nucleus, located in the brainstem, stimulate the secretion of prolactin from serotonergic nerve terminals in the hypothalamus through activation of serotonergic receptors. Hypothalamic dopaminergic neurones that secrete dopamine into the pituitary portal vessels also tonically inhibit the secretion of prolactin. Significant increases in prolactin (which peak at the point of exhaustion) are evident during exercise in the heat that leads to an intolerable thermoregulatory strain. This suggests alterations in central serotonergic and dopaminergic activity, in response to the increased core temperature, that could contribute to the reduced motivation to continue exercise, and subsequently fatigue, in the heat. The prolactin responses at exhaustion were also significantly related to the core temperature responses at exhaustion in these studies, further supporting a link between thermal limits of athletic performance and prolactin levels.

Another study compared the prolactin and the cardiovascular responses at identical core temperatures during an active (exercise) heating trial and a passive (water immersion) heating trial. The passive heating trial was used in this study to obtain similar core temperatures to those achieved in an active (exercise) heat stress but with different cardiovascular responses owing to the absence of exercise. This design provided an insight into the mechanisms of prolactin release and an indication of central serotonergic and dopaminergic activity relating to central fatigue during exercise-induced hyperthermia. The specific hypothesis tested was that peripheral blood flow displacement, and an attendant drop in arterial blood pressure and therefore cerebral blood flow, stimulates the release of prolactin during exercise in hot conditions. The study subjects sat and rested on the cycle ergometer for the same amount of time and under the same conditions as in the active exercise trial with no change in core temperature. In addition, subjects were also immersed

in the water bath at a thermoneutral temperature for the same duration as the passive heat stress and no change in core temperature was evident. In both of these control trials no change in prolactin was observed, indicating that being required to be seated on a cycle ergometer or in a water bath, as in the present study, do not, per se, cause changes in prolactin. In addition, the thermal discomfort ratings at the end of both forms of heating in the present study indicated that the subjects terminated their trials at the point when thermal discomfort was almost maximal. In conclusion, active (exercise-induced) and passive hyperthermia that invoked identical core temperatures yielded similar prolactin responses. This was despite different cardiovascular responses to the two forms of body heating. **These results suggest that increases in core temperature, not alterations in peripheral blood flow and blood pressure, provide the key stimulus for prolactin release, which may be a marker of central serotonergic and dopaminergic activity relating to central fatigue during exercise in hot conditions.**

Although the present study did not include sequential blood sampling during the recovery period, the elevation of plasma prolactin has been shown to return to the baseline within 1 h after exercise (23, 27). It is unknown how long the increased prolactin-receptor expression on B lymphocytes continues. If the immunostimulatory function of prolactin and its half-life of 15–20 min are considered, exercise-induced elevation of prolactin is consistent with a promotion in immune cell function. Animal studies by Ortega et al. (35) support this possible relationship. They demonstrated that acute aerobic exercise increased the plasma prolactin concentration as well as the phagocytic activity of macrophages in mice. Furthermore, previous studies with humans have demonstrated that there is an enhancement of antibody production or serum Ig concentrations in response to acute aerobic exercise (19, 32, 36). Although these investigators did not analyze prolactin, its presence is consistent with the elevations of serum immunoglobulin in response to acute exercise. Because of its immune-enhancing property, prolactin has been considered as a therapeutic agent for immune-deficient patients, including those undergoing bone marrow transplant and radiation/chemotherapy (4, 39). In addition, hormone therapy may

be useful for slowing a decline in the immune and other systems during aging (17, 29). Although most animal studies have investigated prolactin in connection with surgical, chemical, and mechanical stress, the present study demonstrates a possible relationship between prolactin and human immune cells in response to exercise. Thus one of the positive effects of physical exercise may include an increase in endogenous prolactin especially for patients with a deficiency in the immune system. In summary, the present study demonstrated that acute aerobic exercise elevated plasma prolactin concentrations and the total number of circulating B lymphocytes expressing prolactin receptor. In addition, there was an increase in total prolactin-receptor expression per B lymphocyte in response to exercise. Furthermore, B-cell prolactin-receptor expression was positively correlated with plasma prolactin concentrations. Thus this study supports the idea that physical exercise may enhance the interaction between human immune target cells and prolactin, a hormone capable of stimulating the immune function. The knowledge of exercise-induced immunoregulation may facilitate our total understanding of the intercommunication between endocrine and immune systems general, these studies were based on exogenous administration of prolactin (30), the use of surgical procedures (37), or the use of chemicals such as haloperidol to increase prolactin (28). In only one published study were the effects of prolactin elevation on prolactin-receptor expression by human leukocytes reported (26). This study indicated no significant difference in prolactin-receptor expression between hyperprolactinemic and normal subjects. However, chronic prolactin elevation at rest is likely different from an acute increase in prolactin through physical exercise by the healthy population in the present study. Moreover, Clodi et al. (11), who examined cytokine production and NK cell activity of hyperprolactinemic patients with pituitary tumors, found that chronically elevated serum prolactin concentrations induced adaptation and abolished the acute immunostimulatory effects of prolactin. Therefore, acute prolactin elevation may increase prolactin-receptor expression by immune cells even though persistent acute

prolactin elevation may not. In the present study, there was a significant positive correlation between plasma prolactin concentrations and total prolactin-receptor expression per B lymphocyte, although correlational analysis cannot establish causation. Another possible mechanism of exercise-induced prolactin-receptor expression may be mediated through cortisol, which is known to be immunosuppressive (13). High-intensity exercise is well known to increase cortisol concentrations (22).

## Core Temperature Is the Primary Factor in Exhaustion or Fatigue

“Normal” body temperature varies from person to person and also depends upon the place in the [body](#) at which the measurement is made, the time of day, as well as the activity level of the person. Nevertheless, commonly mentioned typical values are as follows:

Oral (under the tongue):  $36.8 \pm 0.4$  °C ( $98.2 \pm 0.72$  °F)

Internal ([rectal](#), [vaginal](#)): 37.0 °C (98.6 °F)

Different parts of the body have different temperatures. Rectal and vaginal measurements taken directly inside the body cavity are typically slightly higher than oral measurements, and oral measurements are somewhat higher than skin measurements. Other places, such as under the arm or in the ear, produce different typical temperatures. Although some people think of these averages as representing the normal or ideal temperature, a wide range of temperatures has been found in healthy people. The body temperature of a healthy person varies during the day by about 0.5 °C (0.9 °F) with lower temperatures in the morning and higher temperatures in the late afternoon and evening, as the body's needs and activities change. Other circumstances also affect the body's temperature. The core body temperature of an individual tends to have the lowest value in the second half of the sleep cycle; the lowest point, called the [nadir](#), is one of the primary markers for [circadian rhythms](#). The body temperature also changes when a person is hungry, sleepy, sick, or cold. Core temperature, also called core body temperature, is the operating [temperature](#) of an [organism](#), specifically in deep structures of the body such as the [liver](#), in comparison to temperatures of peripheral tissues. Core temperature is normally maintained within a narrow range so that essential enzymatic reactions can occur. Significant core temperature elevation ([hyperthermia](#)) or depression ([hypothermia](#)) that is prolonged for more than a brief period of time is incompatible with human life.



Temperature examination in the [rectum](#) is the traditional [gold standard](#) measurement used to estimate core temperature (oral temperature is affected by hot or cold drinks and mouth-breathing). Rectal temperature is expected to be approximately one Fahrenheit degree higher than an oral temperature taken on the same person at the same time. Ear thermometers measure eardrum temperature using [infrared](#) sensors. The blood supply to the [tympanic membrane](#) is shared with the [brain](#). However, this method of measuring body temperature is not as accurate as rectal measurement and has a low sensitivity for fevers, missing three or four out of every ten fevers in children.<sup>[15]</sup> Ear temperature measurement may be acceptable for observing trends in body temperature but is less useful in consistently identifying fevers.

Until recently, direct measurement of core body temperature required surgical insertion of a probe, so a variety of indirect methods have commonly been used. The [rectal](#) or [vaginal](#) temperature is generally considered to give the most accurate assessment of core body temperature, particularly in hypothermia. In the early 2000s, ingestible [thermistors](#) in [capsule](#) form were produced, allowing the temperature inside the [digestive tract](#) to be transmitted to an external receiver; one study found that these were comparable in accuracy to rectal temperature measurement.

The ability of humans to endure passive heat stress or the heat generated by active muscle during endurance exercise is limited by the maximum core temperature achieved during the exercise. Fatigue generally coincides with core temperatures ranging from 38 degrees C. to 40 degrees C. (104 degrees F.) (McArdle @ page 649). This temperature range reflects a critical high body temperature that impairs muscle activation directly from a high brain temperature that decreases the central drive to continue exercising. (McArdle @ page 649). Animal studies have determined, for example, that in exercising rats, exhaustion is reached at a core temperature of about 42°C. (107.6 degrees F.), regardless of the temperatures at the initiation of exercise.

Published studies provide clear support for the concept that **a critical internal temperature limits both moderate and strenuous exercise in the heat**.<sup>(1)</sup> A number of reports have linked internal

temperature to impaired physical performance in the heat in humans and in animals. Caputa et al. (2) reported that reduced running performance occurred when  $T_{hyp}$  reached 42.0–42.9°C in exercising goats. Furthermore, cheetahs cease running when their core temperature reaches 40.5°C (21), whereas domestic dogs (beagles) reach exhaustion at  $T_{rec}$  between 41.7 and 42.2°C (24). Endurance-trained humans exercising in the heat become exhausted at between 39.7 and 40.3°C (6, 15). These studies have primarily used two different strategies to examine the question of whether a critical, exercise-limiting internal temperature exists. The first method is by altering the rate of heating during exercise by varying ambient temperature during exercise (6, 12, 24). The second method involves the use of heating (or cooling) before exercise to alter the initial temperature. When environmental **heating** is used as a preheating modality, the number of levels of preheating is limited. This is because the length of time required to induce a significant elevation in baseline temperature eventually leads to the introduction of confounding variables such as dehydration, electrolyte imbalances, and cardiovascular drift that hasten fatigue during exercise.

Human studies have also concluded that the physical endurance for exercise in hot, dry environments appears to be limited by the attainment of a critical level of core temperature...reducing motivation (Bruck & Olschewski, 1987). High core temperature, and not circulatory failure or metabolic depletion, is the critical factor in heat stress, both before and after acclimation (Nielsen, B., Hales, J.R., et al (1993); Fink, et. al (1975), Kozlowski, et. al. 1985)). Different aerobic fitness levels often result in slightly different core temperature at exhaustion tolerance levels, but experts believe this may result from the familiarization of regular aerobic training and exposure to higher body temperatures on a daily basis. (Selkirk, G. and McLellan, T., (2001).

It has been proposed that a critical sublethal temperature exists beyond which physical activity is not possible; such a mechanism is hypothesized to protect animals from reaching a lethal level of hyperthermia. Studies designed to assess lethality of exertional heat stress have

demonstrated that rats will run to the point of heatstroke leading to death. However, it is not temperature alone that determines the lethality of heat stress, but rather the thermal load, which is determined by the level of hyperthermia and the duration for which it is sustained (4, [11](#)). We calculated the thermal load encountered by our rats during exercise by the method of Fruth and Gisolfi (4) and found similar values ( $37.4\text{--}43.8^{\circ}\text{C} \cdot \text{min}$ ) after all treatments. This range of values is below the lethal thermal load reported for untrained rats by Fruth and Gisolfi. Thus our rats became fatigued before they reached a lethal thermal load. In the present study, as well as in a previous investigation ([23](#)), we found that rats always became too fatigued to run before lethal thermal loads were encountered. Studies that have exercised rats to lethality have intentionally designed the exercise protocol to maximize the thermal load encountered by the animals. This has been accomplished by either running rats under a work-rest paradigm at a low level of exercise ([11,19](#)) or by ramping up exercise intensity and environmental temperature during the course of the session (4). Interestingly, in this last study (4), fatigue occurred at a  $T_{\text{rec}}$  ( $42.4^{\circ}\text{C}$ ) similar to the one we report. In contrast to our study, however, Fruth and Gisolfi reported 100% mortality. The difference between the studies relates to the thermal load. Although exhaustion was reached at the same temperature in both studies, Fruth and Gisolfi used ramped-up temperature and TM speed to produce a thermal load threefold greater than that achieved in our investigation. This is important because it suggests that, although there may be a critical temperature beyond which exercise cannot continue in the heat, it is not sensitive to thermal load and thus cannot protect against thermal damage or lethality under all circumstances. Another condition in which the critical temperature cannot protect against lethality can occur during low-level exercise in the heat. Under these conditions, a lethal thermal load can occur before the critical temperature for cessation of exercise is reached ([11, 19](#)).

Although this investigation provides evidence for the existence of a limiting body temperature during exercise in the heat, it is not clear how these limits are controlled. Exercise ceased at a similar  $T_{\text{hyp}}$  and  $T_{\text{rec}}$  independent of the level of preheating; thus it cannot be determined from the present study whether fatigue is related to elevated brain or core temperature. It was not

possible to measure metabolic indicators associated with fatigue (e.g., blood glucose, lactate, or muscle glycogen concentrations) because of the repeated-measures design of the study. If these factors were involved, however, it is unlikely that their influence would coincide with the same internal temperatures across treatments. In addition, exercise after medium and high was only 19.4 and 10.7 min, respectively, which is too short a time period in which to expect significant substrate depletion. Furthermore, it has been demonstrated in humans exercising in the heat that exhaustion occurs before significant decline in muscle glycogen and blood glucose concentrations or increases in muscle and blood lactate concentrations ([14](#), [15](#), [16](#)). A recent investigation in humans ([16](#)) has linked accumulation of IMP and  $\text{NH}_3$  with fatigue in the heat. Studies in racehorses have led to the postulation that fatigue in the heat may also be due to metabolic dysfunction precipitated by oxidative stress ([13](#)). Although we cannot rule out these possibilities in the present investigation, they appear unlikely because of the fact that fatigue occurred at nearly the identical temperature after all treatments. Dehydration was also not a factor, given that the amount of weight lost during exercise does not reflect a level of dehydration associated with fatigue (Table [4](#)).

It has been suggested that fatigue during exercise in the heat is related to a diminished central drive ([1](#)). The hypothalamus has been shown to be involved in a myriad of thermoregulatory responses and behaviors [for review, see Gordon, Ref. ([9](#))]. It would thus appear to be a likely candidate for limiting exercise in the heat. Caputa et al. ([2](#)) used intravascular heat exchangers to selectively heat and cool the brain and trunk independently in exercising goats. The results of these experiments have demonstrated that exhaustion is reached when  $T_{\text{hyp}}$  reaches 42.0–42.9°C if trunk temperature is maintained at 40.0°C. In contrast, a trunk temperature of over 43.5°C was required before exercise performance was affected. This suggests central involvement in mediating fatigue. The postural extension response, used by Fuller et al. ([5](#)) as their operational definition of fatigue, has been clearly demonstrated to occur in response to warming of the preoptic area of the anterior hypothalamus ([18](#)). Thus fatigue during volitional exercise in rats is most likely controlled by the hypothalamus. However, it is not clear whether

the hypothalamus mediates fatigue under the more stringent criteria used in the present study. Although this study provides strong evidence for the existence of a critical temperature that limits exercise in the heat, additional investigations are required to determine where the locus of control resides. In addition, it remains to be determined whether temperature per se is the critical variable or whether the temperature just coincides with the limiting variable.

In summary, this study clearly demonstrates that exhaustion during exercise in the heat in rats occurs at a critical internal temperature level regardless of the initial levels of temperature. By using MW technology, we were able to set initial temperature at various levels rapidly, thereby avoiding confounding factors inherent to more familiar modalities of preexercise heating.

Because the temperatures at exhaustion were virtually identical, whereas the run times to exhaustion were correlated with the initial temperatures, this study provides strong evidence that exercise is limited by the attainment of a critical internal temperature.

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Walters, T.J., Ryan, K.L., Tate, L.M., Mason, P.A., Exercise in the heat is limited by a critical internal temperature, Journal of Applied Physiology Published 1 August 2000 Vol. 89 no. 2, 799-806 DOI:

## Heat Shock Proteins (HSPs) and BDNF: What's the Difference?

Both brain-derived neurotrophic factor, also known as **BDNF**, and heat shock proteins (**HSPs**) are proteins, but each has a different...

- A. Proteins:** Proteins are large [biomolecules](#), or [macromolecules](#), that are made up of one or more long chains of [amino acid residues](#). Proteins perform many important functions within living [organisms](#). The functions of proteins include the following:
1. [Catalyzing metabolic reactions](#) (enzyme functions);
  2. [DNA replication](#);
  3. [Responding to stimuli](#); and
  4. Transporting molecules from one location to another.

Proteins differ from one another primarily in their **sequence of amino acids**, which is dictated by the [nucleotide sequence](#) of their [genes](#). The nucleotide sequences usually result in [protein folding](#) into a specific [three-dimensional structures](#) that determine their activities.

A **linear chain** of amino acid residues is called a [polypeptide](#). Proteins always contain at least one **long** polypeptide. **Short** polypeptides, which usually contain less than 20-30 residues, are rarely considered to be proteins and are commonly called [peptides](#). The individual amino acid residues are bonded together by [peptide bonds](#) and adjacent amino acid residues. The [sequence](#) of amino acid residues in a protein is defined by the [sequence](#) of a [gene](#), which is encoded in the [genetic code](#). In general, the genetic code specifies 20 standard amino acids; however, in certain organisms the genetic code can include [selenocysteine](#) and—in certain [archaea](#)—[pyrrolysine](#). Shortly after or even during synthesis, the residues in a protein are often chemically modified by [posttranslational modification](#), which alters the physical and chemical properties, folding, stability, activity, and ultimately, the function of the proteins. Sometimes proteins structural or mechanical functions, such as [actin](#) and [myosin](#) in muscle and the proteins in the [cytoskeleton](#), which form a system of [scaffolding](#) that maintains cell shape. Other proteins are important in [cell signaling](#), [immune responses](#), [cell adhesion](#), and the [cell cycle](#). Proteins are also necessary in animals' diets, since animals cannot [synthesize](#) all the amino acids they need and must obtain [essential amino acids](#) from food. Through the process of [digestion](#), animals break down ingested protein into free amino acids that are then used in metabolism.

**B. Heat shock proteins (HSPs):** HSPs are a family of proteins that are produced by [cells](#) in response to exposure to stressful conditions. They were first described in relation to [heat shock](#),<sup>[1]</sup> but are now known to also be expressed during other stresses including exposure to cold,<sup>[2]</sup> UV light,<sup>[3]</sup> and during wound healing or tissue remodeling.<sup>[4]</sup> Many members of this group perform [chaperone](#) function by stabilizing new proteins to ensure correct folding or by helping to refold proteins that were damaged by the cell stress.<sup>[5]</sup> This increase in expression is [transcriptionally](#) regulated. The dramatic [upregulation](#) of the heat

shock proteins is a key part of the [heat shock response](#) and is induced primarily by [heat shock factor](#) (HSF).<sup>[6]</sup> HSPs are found in virtually all living organisms, from [bacteria](#) to [humans](#). Production of high levels of heat shock proteins can also be triggered by exposure to different kinds of environmental [stress](#) conditions, such as [infection](#), [inflammation](#), exercise, exposure of the cell to toxins ([ethanol](#), [arsenic](#), [trace metals](#), and [ultraviolet](#) light, among many others), [starvation](#), [hypoxia](#) ([oxygen](#) deprivation), nitrogen deficiency (in plants), or water deprivation. As a consequence, the heat shock proteins are also referred to as **stress proteins** and their [upregulation](#) is sometimes described more generally as part of the **stress response**.<sup>[12]</sup>

The mechanism by which heat-shock (or other environmental stressors) activates the heat shock factor has been determined in bacteria. During heat stress outer membrane proteins (OMPs) do not fold and cannot insert correctly into the outer membrane. They accumulate in the periplasmic space. These OMP's are detected by DegS, an inner membrane protease, that passes the signal through the membrane to the sigmaE transcription factor.<sup>[13]</sup> However, some studies suggest that an increase in damaged or abnormal proteins brings HSPs into action.

Some bacterial heat shock proteins are upregulated via a mechanism involving RNA thermometers such as the [FourU thermometer](#), [ROSE element](#) and the [Hsp90 cis-regulatory element](#).<sup>[14]</sup> Several heat shock proteins function as intra-cellular [chaperones](#) for other proteins. They play an important role in protein-protein interactions such as folding and assisting in the establishment of proper protein [conformation](#) (shape) and prevention of unwanted protein aggregation. By helping to stabilize partially unfolded proteins, HSPs aid in transporting proteins across membranes within the cell.<sup>[15][16]</sup>

Some members of the HSP family are expressed at low to moderate levels in *all* organisms because of their essential role in protein maintenance. Heat-shock proteins also occur under non-stressful conditions, simply "monitoring" the cell's proteins. Some examples of their role as "monitors" are that they carry old proteins to the cell's "recycling bin" ([proteasome](#)) and they help newly synthesized proteins fold properly. These activities are part of a cell's own repair system, called the "cellular stress response" or the "heat-shock response".

**C. Brain-derived neurotrophic factor (BDNF):** BDNF is also a protein. In humans, BDNF is encoded by the *BDNF gene*.<sup>[3][4]</sup> BDNF is a member of the [neurotrophin](#) family of growth factors, which are related to the canonical [Nerve Growth Factor](#). [Neurotrophic factors](#) are found in the [brain](#) and the periphery. BDNF acts on certain [neurons](#) of the [central nervous system](#) and the [peripheral nervous system](#), helping to support the survival of existing neurons, and encourage the growth and [differentiation](#) of new neurons and [synapses](#).<sup>[5][6]</sup> In the brain, it is active in the [hippocampus](#), [cortex](#), and [basal forebrain](#)—areas vital to [learning](#), [memory](#), and higher thinking.<sup>[7]</sup> It is also expressed in the retina, motor neurons, the kidneys, saliva, and the prostate.<sup>[8]</sup>

BDNF itself is important for [long-term memory](#).<sup>[9]</sup> Although the vast majority of neurons in the [mammalian](#) brain are formed prenatally, parts of the adult brain retain the ability to grow new neurons from neural [stem cells](#) in a process known as [neurogenesis](#). Neurotrophins are chemicals that help to stimulate and control neurogenesis, BDNF being one of the most active.<sup>[10][11][12]</sup> Mice born without the ability to make BDNF suffer developmental defects in the brain and [sensory nervous system](#), and usually

die soon after birth, suggesting that BDNF plays an important role in normal [neural development](#).<sup>[13]</sup> Other important neurotrophins structurally related to BDNF include [NT-3](#), [NT-4](#), and [NGF](#).

BDNF is made in the [endoplasmic reticulum](#) and secreted from dense-core vesicles. It binds [carboxypeptidase E](#) (CPE), and the disruption of this binding has been proposed to cause the loss of sorting of BDNF into dense-core vesicles. The [phenotype](#) for BDNF knockout mice can be severe, including postnatal lethality. Other traits include sensory neuron losses that affect coordination, balance, hearing, taste, and breathing. Knockout mice also exhibit cerebellar abnormalities and an increase in the number of sympathetic neurons.<sup>[14]</sup>

[Certain types of physical exercise](#) have been shown to markedly (threefold) increase BDNF synthesis in the human brain, a phenomenon which is partly responsible for exercise-induced neurogenesis and improvements in cognitive function.<sup>[15][16][17][18]</sup> Niacin appears to upregulate BDNF and [tropomyosin receptor kinase B](#) (TrkB) expression as well.<sup>[19]</sup>

Mechanism of action<sup>[edit]</sup>

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BDNF binds at least two receptors on the surface of cells that are capable of responding to this growth factor, [TrkB](#) (pronounced "Track B") and the [LNGFR](#) (for *low-affinity nerve growth factor receptor*, also known as p75).<sup>[20]</sup> It may also modulate the activity of various neurotransmitter receptors, including the [Alpha-7 nicotinic receptor](#).<sup>[21]</sup> BDNF has also been shown to interact with the [reelin](#) signaling chain.<sup>[22]</sup> The expression of reelin by [Cajal-Retzius cells](#) goes down during development under the influence of BDNF.<sup>[23]</sup> The latter also decreases reelin expression in neuronal culture.



## **White Paper: Hyperthermic Conditioning and Alzheimer's Disease**

Alzheimer's disease (AD) is the most common form of dementia. It primarily affects adults over the age of sixty. AD affects the brain by destroying neurons—the basic components of the brain. Neurons are the chief type of cell destroyed by Alzheimer's disease. Alzheimer's disease leads to nerve cell death and tissue loss throughout the brain. Over time, the brain shrinks dramatically, affecting nearly all its functions. The real work of the brain goes on in individual cells. An adult brain contains about 100 billion nerve cells, or neurons, with branches that connect at more than 100 trillion points. Scientists call this dense, branching network a "neuron forest." Signals traveling through individual nerve cells as tiny electrical charges move through this dense neuron forest to form the basis of memories, thoughts, and feelings. Nerve cells connect to one another at **synapses**. When a charge reaches a synapse, it may trigger release of tiny bursts of chemicals called **neurotransmitters**. The neuro-transmitters travel across the synapse, carrying signals to other cells. Scientists have identified dozens of neurotransmitters. Alzheimer's disease disrupts both the way electrical charges travel within cells and the activity of neurotransmitters.

In advanced AD, massive cell loss changes the whole brain as follows:

1. The **cortex shrivels up**, damaging areas involved in thinking, planning and remembering;
2. Shrinkage is especially severe in the **hippocampus**, an area of the cortex that plays a key role in formation of new memories;
3. **Ventricles** (fluid-filled spaces within the brain) grow larger.

Many of the devastating effects of Alzheimer's disease are visible when brain tissue is examined under the microscope. Alzheimer's tissue has many fewer nerve cells and synapses than a healthy brain. In addition, plaques (abnormal clusters of protein fragments), build up between nerve cells. Finally, **dead and dying nerve cells contain** *tangles*, which are made up of twisted strands of another protein.

Scientists are not absolutely sure what causes cell death and tissue loss in the Alzheimer's brain, but plaques and tangles are prime suspects.

A. Amyloid Plaques: Plaques form when protein pieces called beta-amyloid clump together (beta-amyloid comes from a larger protein found in the fatty membrane surrounding nerve cells). Beta-amyloid is chemically "sticky" and gradually builds up into plaques. Many experts believe the most damaging form of beta-amyloid may be clumps of small pieces rather than the plaques themselves, because the small clumps may block cell-to-cell signaling at synapses. The clumps may also activate immune system cells that trigger inflammation and devour disabled cells. These plaques are believed to form early in the disease process, before neurons begin to die and the symptoms of memory loss and dementia become apparent.

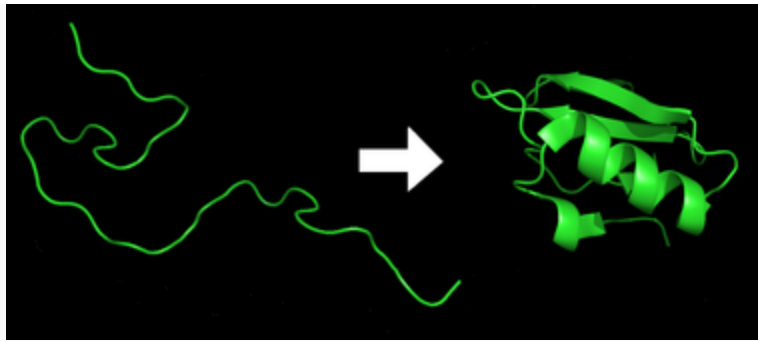
B. Neurofibrillary Tangles: Neurofibrillary refers to tiny filaments or fibers inside nerve cells. Neurofibrillary tangles form when threads of a protein called **tau** begin to twist. Without sufficient support, the twisted cell structure collapses. Tangles ultimately destroy a vital cell transport system made of proteins. In the healthy brain, the cell transport system is **organized in orderly**

[parallel strands](#) like railroad tracks. Food molecules, cell parts and other key materials travel along these "tracks." The tau protein helps the tracks stay straight. When tangles form, the tracks can no longer stay straight. The tracks fall apart and disintegrate, restricting the movement of nutrients and other essential supplies through the brain cells, which eventually die.

### C. Mis-folded Proteins:

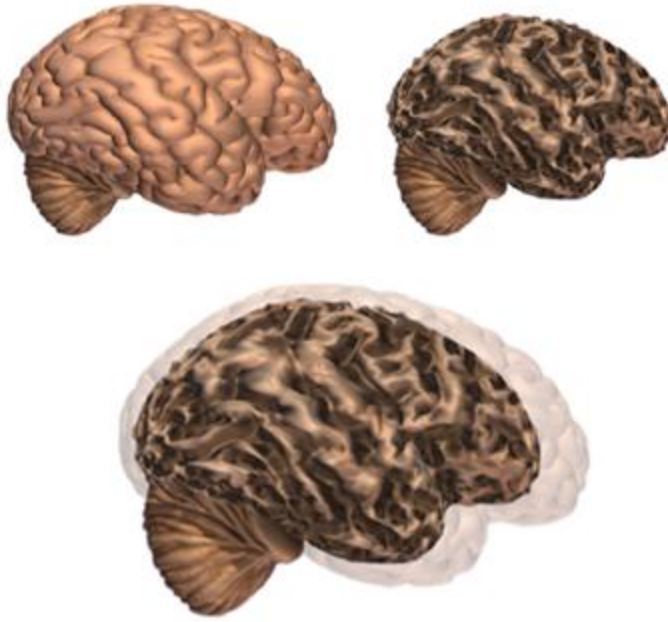
Protein folding is the process by which a [protein](#) structure assumes its functional shape or conformation. It is the physical process by which a [polypeptide](#) folds into its characteristic and functional [three-dimensional structure](#) from [random coil](#). Each [protein](#) exists as an unfolded polypeptide or random coil when translated from a sequence of [mRNA](#) to a linear chain of [amino acids](#). This polypeptide lacks any stable (long-lasting) three-dimensional structure (the left hand side of the first figure). Amino acids interact with each other to produce a well-defined three-dimensional structure, the folded protein (the right hand side of the figure), known as the [native state](#). The resulting three-dimensional structure is determined by the amino acid sequence ([Anfinsen's dogma](#)).<sup>[2]</sup> Experiments<sup>[3]</sup> beginning in the 1980s indicate the codon for an amino acid can also influence protein structure.

The correct three-dimensional structure is essential to function, although some parts of functional proteins [may remain unfolded](#),<sup>[4]</sup> so that [protein dynamics](#) is important. Failure to fold into native structure generally produces inactive proteins, but in some instances misfolded proteins have modified or toxic functionality. Several [neurodegenerative](#) and other [diseases](#) are believed to result from the accumulation of [amyloid](#) fibrils formed by *misfolded* proteins (as shown below). Many [allergies](#) are caused by incorrect folding of some proteins, for the immune system does not produce antibodies for certain protein structures.



Protein before and after folding.

Aggregated proteins are associated with [prion](#)-related illnesses such as [Creutzfeldt-Jakob disease](#), [bovine spongiform encephalopathy](#) (mad cow disease), [amyloid](#)-related illnesses such as [Alzheimer's disease](#) and familial amyloid cardiomyopathy or polyneuropathy, as well as intracytoplasmic aggregation diseases such as Huntington's and Parkinson's disease. These age onset degenerative diseases are associated with the aggregation of misfolded proteins into insoluble, extracellular aggregates and/or intracellular inclusions including cross-beta sheet [amyloid fibrils](#). While it is not completely clear whether the aggregates are the cause or merely a reflection of the loss of protein homeostasis, the balance between synthesis, folding, aggregation and protein turnover, the recent [European Medicines Agency](#) approval of [Tafamidis](#) or Vyndaqel (a kinetic stabilizer of tetrameric transthyretin) for the treatment of the transthyretin amyloid diseases suggests that it is the process of amyloid fibril formation and not the fibrils themselves that causes the degeneration of post-mitotic tissue in human amyloid diseases.<sup>[18]</sup> Misfolding and excessive degradation instead of folding and function leads to a number of [proteopathy](#) diseases such as antitrypsin-associated [emphysema](#), [cystic fibrosis](#) and the [lysosomal storage diseases](#), where loss of function is the origin of the disorder. While protein replacement therapy has historically been used to correct the latter disorders, an emerging approach is to use [pharmaceutical chaperones](#) to fold mutated proteins to render them functional.

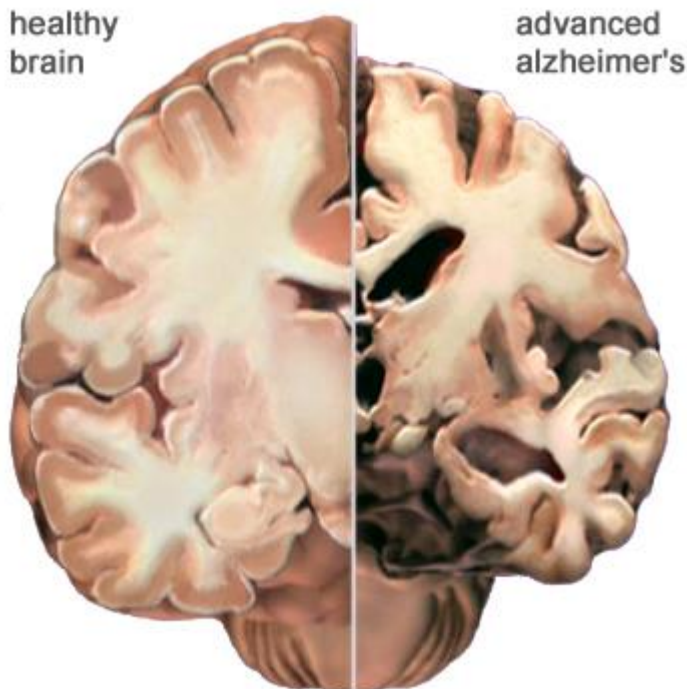


Alzheimer's disease leads to nerve cell death and tissue loss throughout the brain. Over time, the brain shrinks dramatically, affecting nearly all its functions.

These images show:

- **A brain without the disease**
- **A brain with advanced Alzheimer's**

More brain changes



Here is another view of how massive cell loss changes the whole brain in advanced Alzheimer's disease. This slide shows a crosswise "slice" through the middle of the brain between the ears.

In the Alzheimer's brain:

- The **cortex shrivels up**, damaging areas involved in thinking, planning and remembering.
- Shrinkage is especially severe in the **hippocampus**, an area of the cortex that plays a key role in formation of new memories.
- **Ventricles** (fluid-filled spaces within the brain) grow larger.

## **A. EXERCISE AND ALZHEIMER'S DISEASE**

A number of recent clinical trials have shown numerous benefits for AD sufferers from physical exercise. Physical activity was shown to improve mood, memory and ability to think for participants in three new studies. One study found that intense aerobic exercise improves blood flow to key areas of the brain, and appears to reduce the tau protein tangles that are a hallmark of Alzheimer's disease. According to one of the study leaders, Laura Baker (cognitive neuroscientist at Wake Forest School of Medicine in Winston-Salem, N.C.):

"Blood flow decreases in those areas for all of us with age, and yet exercise increased it." "It seems to me we're changing aging-related effects, and we may be changing Alzheimer's-related effects, both with exercise."

The three new studies "give us information about living better with the disease," said Heather Snyder, director of medical and scientific operations for the Alzheimer's Association. "Physical exercise is potentially beneficial to people who are living with Alzheimer's today," Snyder said. "Even once you have cognitive impairment, there's still a benefit to physical activity."

Earlier research has shown that exercise can improve the ability to think in healthy adults, so Baker and her colleagues turned to people with mild impairment to see if physical activity would help them, too. The 65 people in Baker's study were 55 to 89 years old and had not been exercising beforehand. They also had prediabetes, which can increase risk of developing Alzheimer's disease. Participants were randomly assigned to one of two groups for six months. The first group performed stretching exercises that did not raise their heart rate much, while the second group had to perform at least 45 minutes of high-intensity aerobics four times a week.

The aerobics group had to stay within 75 percent to 85 percent of their maximum heart rate for at least 30 minutes of their workout, which most often took place on a treadmill. "For our typical 70-year-old, that means a heart rate of at least 130 beats per minute," Baker said. Ninety-two

percent of people stuck to the exercise program, and wound up with improved fitness and better blood sugar levels, researchers found.

More important, MRI brain scans revealed that blood flow had significantly increased to the memory and processing centers of participants' brains, with a corresponding improvement in their ability to plan, organize and pay attention. Tests using cerebro-spinal fluid samples drawn from the patients also showed a significant reduction in tau protein tangles, with the effect most pronounced in those older than 70. "These findings are important because they strongly suggest a potent lifestyle intervention such as aerobic exercise can impact Alzheimer's-related changes in the brain," Baker said. "No currently approved medication can rival these effects."

In another clinical trial, 200 people between ages 50 and 90 with Alzheimer's were randomly assigned to either an aerobic exercise program or a control group that performed no extra exercise. The folks who exercised were asked to reach a target intensity of 70 percent to 80 percent of their maximum heart rate. The Danish researchers found that those who exercised suffered from fewer mood problems such as anxiety, irritability and depression. The people who exercised most often and most vigorously also achieved significant improvements in mental speed and attention.

The third clinical trial took place in Canada and involved 71 people between ages 56 and 96 who had suffered ministrokes, diminishing their ability to think and remember. Half were assigned to a group that took part in regular aerobics classes. The researchers found that participants who took aerobics significantly improved their memory and selective attention, compared with those not asked to exercise regularly.

## **B. 2/3rds of AMERICA'S ALZHEIMER'S SUFFERERS ARE WOMEN**

1. Two-thirds of Americans suffering from Alzheimer's are women.
2. Women with memory problems that may signal early Alzheimer's descend into dementia twice as fast as men, according to recent research.

3. Women in their 60s are twice as likely to develop Alzheimer's as they are to get breast cancer (Alzheimer's Association).

Researchers suspect that the pronounced differences between men and women and Alzheimer's has something to do with the biology of the brain. Experts say it may relate to the complicated interaction between genetics, hormones and the way the brain develops. Studies have found a number of differences between gender, including findings that women are more likely than men to suffer from depression (a known risk factor for Alzheimer's disease) and that women are more vulnerable to stress (also a risk factor for Alzheimer's).(1) It has also been found women have more of the brain-clogging protein called **amyloid** that is a hallmark of Alzheimer's.

A recent study evaluated 1,000 people: 273 normal people, 557 with mild cognitive impairment and 145 who had diagnosed Alzheimer's. The study confirmed that women have more amyloid in the brain than men even when other factors were adjusted for.(1)

(1) Presented along with a series of studies presented July 21, 2015 at the Alzheimer's Association International Conference (Dr. Michael Weiner, et al, University of California at San Francisco).

### **C. BDNF AND ALZHEIMER'S DISEASE**

Brain-derived neurotrophic factor (BDNF) has been described by some experts as "Miracle-Gro for the brain" due to its properties of strengthening existing neurons and helping new neurons grow. Recent evidence suggests that amyloid-beta associated neurotoxicity may be a consequence of a **deficiency** of BDNF. Several studies indicate that the cortex and hippocampus, areas of the brain associated with learning and memory, exhibit both extensive amyloid pathology and decreased levels of BDNF in AD ([Phillips et al., 1991](#); [Connor et al., 1997](#); [Ferrer et al., 1999](#); [Hock et al., 2000](#); [Holsinger et al., 2000](#); [Garzon et al., 2002](#); [Peng et al., 2005](#)). Interestingly, BDNF protein levels are significantly decreased in preclinical and early stages of AD, and this reduction correlates with clinical neuropsychological scores ([Peng et al.,](#)



[2005](#)). Since BDNF is critical for neuronal survival and function ([Siegel and Chauhan, 2000](#); [Mufson et al., 2007](#)) and for synaptic plasticity and learning and memory ([Korte et al., 1995](#); [Patterson et al., 1996](#); [Lu, 2003](#); [Bramham and Messaoudi, 2005](#); [Nagahara et al., 2009](#)), which are compromised in AD, it is important to understand which A $\beta$  species drive the reduction of BDNF in AD. *In vitro* data demonstrate that soluble forms of A $\beta$  decrease BDNF mRNA expression and compromise BDNF intracellular signaling in both primary rat neurons and human neuroblastoma cells ([Tong et al., 2001,2004](#); [Garzon and Fahnestock, 2007](#)). Therefore, amyloid-induced neurodegeneration may be a consequence of reduced BDNF. However, whether A $\beta$  assemblies downregulate BDNF *in vivo*, and which A $\beta$  assembly state is responsible for BDNF downregulation, have not been elucidated. In this study, we measured levels of BDNF mRNA and A $\beta_{42}$ /A $\beta_{40}$  ratios and characterized the state of A $\beta$  in three different transgenic mouse models of AD ([Table 1](#)) containing mutations in *APP*, two of these in combination with *PS-1* mutations, and in a mouse model of Down syndrome (segmental trisomy 16) containing an additional copy of *App*.

[Neurobiol Dis.](#) 2008 Sep;31(3):316-26. doi: 10.1016/j.nbd.2008.05.012. Epub 2008 Jul 15.

### **Protective effect of BDNF against beta-amyloid induced neurotoxicity in vitro and in vivo in rats.**

[Arancibia S<sup>1</sup>](#), [Silhol M](#), [Moulière F](#), [Meffre J](#), [Höllinger I](#), [Maurice T](#), [Tapia-Arancibia L](#).

We examined the potential protective effect of BDNF against beta-amyloid-induced neurotoxicity in vitro and in vivo in rats. In neuronal cultures, BDNF had specific and dose-response protective effects on neuronal toxicity induced by Abeta(1-42) and Abeta(25-35). It completely reversed the toxic action induced by Abeta(1-42) and partially that induced by Abeta(25-35). These effects involved TrkB receptor activation since they were inhibited by K252a. Catalytic BDNF receptors (TrkB.FL) were localized in vitro in cortical neurons (mRNA and protein). In in vivo experiments, Abeta(25-35) was administered into the indusiumgriseum or the third ventricle and several parameters were measured 7 days later to evaluate potential Abeta(25-35)/BDNF interactions, i.e. local measurement of BDNF release, number of hippocampal hilar cells expressing SRIH mRNA and assessment of the corpus callosum damage (morphological examination, pyknotic nuclei counting and axon labeling with anti-MBP antibody). We conclude that BDNF possesses neuroprotective properties against toxic effects of Abeta peptides.

[J Neurochem.](#) 2004 Feb;88(4):1010-8.

**Regulation of beta-amyloid precursor protein expression by brain-derived neurotrophic factor involves activation of both the Ras and phosphatidylinositol 3-kinase signalling pathways.**

[Ruiz-León Y<sup>1</sup>](#), [Pascual A.](#)

Brain-derived neurotrophic factor (BDNF) stimulates beta-amyloid precursor protein (APP) promoter activity by a Ras-dependent mechanism in TrkB-expressing SH-SY5Y cells. To determine the signalling pathways involved in the BDNF-induced response, we have analysed the ability of TrkB mutated forms to mediate promoter stimulation. Brain-derived neurotrophic factor causes a significant induction of promoter activity and mutation K540R in the active site of TrkB completely abolishes the neurotrophin-induced response. A substitution of the Y484 residue by phenylalanine, which blocks binding of Shc, reduces the activation of APP promoter by BDNF by approximately 50% whereas mutation Y785P, which blocks binding of phospholipase C gamma, does not affect the response. In addition, the phosphatidylinositol 3-kinase (PI3K)-specific inhibitors wortmannin and LY294002 reduced BDNF-induced activation. In agreement with a participation of both Ras/MAPK- and PI3K/Akt-mediated mechanisms, transient expression of constitutive active forms of Ras, PI3K and other components of both signalling pathways led to a significant increase of APP promoter activity. Furthermore, the stimulation of the APP promoter by BDNF was completely precluded by expression of dominant-negative forms of Ras and PI3K effectors. Taken together, our results suggest that simultaneous activation of at least two signalling pathways, Ras/MAPK and PI3K/Akt, is necessary to mediate a full activation of the APP promoter by BDNF.

[J Neurosci.](#) 2009 Jul 22;29(29):9321-9. doi: 10.1523/JNEUROSCI.4736-08.2009.

**Decreased brain-derived neurotrophic factor depends on amyloid aggregation state in transgenic mouse models of Alzheimer's disease.**

[Peng S<sup>1</sup>](#), [Garzon DJ](#), [Marchese M](#), [Klein W](#), [Ginsberg SD](#), [Francis BM](#), [Mount HT](#), [Mufson EJ](#), [Salehi A](#), [Fahnestock M.](#)

Downregulation of brain-derived neurotrophic factor (BDNF) in the cortex occurs early in the progression of Alzheimer's disease (AD). Since BDNF plays a critical role in neuronal survival, synaptic plasticity, and memory, BDNF reduction may contribute to synaptic and cellular loss and memory deficits characteristic of AD. In vitro evidence suggests that amyloid-beta (A beta) contributes to BDNF downregulation in AD, but the specific A beta aggregation state responsible for this downregulation in vivo is unknown. In the present study, we examined cortical levels of BDNF mRNA in three different transgenic AD mouse models harboring mutations in APP resulting in A beta overproduction, and in a genetic mouse model of Down syndrome. Two of the three A beta transgenic strains (APP(NLh) and TgCRND8) exhibited significantly decreased cortical BDNF mRNA levels compared with wild-type mice, whereas neither the other strain (APP(swe)/PS-1) nor the Down syndrome mouse model (Ts65Dn) was affected. Only APP(NLh) and TgCRND8 mice expressed high A beta(42)/A beta(40) ratios and larger SDS-stable A beta oligomers (approximately 115 kDa). TgCRND8 mice exhibited downregulation of BDNF transcripts III and IV; transcript IV is also downregulated in AD. Furthermore, in all transgenic mouse strains, there was a correlation between levels of large oligomers, A beta(42)/A beta(40), and severity of BDNF decrease. These data show that the amount and species of A beta vary among transgenic mouse models of AD and are negatively correlated with BDNF levels. These findings also suggest that the effect of A beta on decreased

BDNF expression is specific to the aggregation state of A beta and is dependent on large oligomers.

New clinical studies have shown that BDNF slows cognitive decline in pre-clinical Alzheimer's disease.

*Molecular Psychiatry* advance online publication 7 October 2014; doi: 10.1038/mp.2014.123

*APOE and BDNF polymorphisms moderate amyloid  $\beta$ -related cognitive decline in preclinical Alzheimer's disease*

Y Y Lim<sup>1,2,3</sup>, V L Villemagne<sup>1,4,5</sup>, S M Laws<sup>6,7,8</sup>, R H Pietrzak<sup>9</sup>, P J Snyder<sup>2,3</sup>, D Ames<sup>10,11</sup>, K A Ellis<sup>1,11</sup>, K Harrington<sup>1</sup>, A Rembach<sup>1</sup>, R N Martins<sup>6</sup>, C C Rowe<sup>4,5</sup>, C L Masters<sup>1</sup> and P Maruff<sup>1,12</sup>

Received 19 May 2014; Revised 29 July 2014; Accepted 21 August 2014  
Advance online publication 7 October 2014

## Abstract

Accumulation of  $\beta$ -amyloid (A $\beta$ ) in the brain is associated with memory decline in healthy individuals as a prelude to Alzheimer's disease (AD). Genetic factors may moderate this decline. We examined the role of apolipoprotein E ( $\epsilon 4$  carrier [ $\epsilon 4^+$ ],  $\epsilon 4$  non-carrier [ $\epsilon 4^-$ ]) and brain-derived neurotrophic factor ( $BDNF^{Val/Val}$ ,  $BDNF^{Met}$ ) in the extent to which they moderate A $\beta$ -related memory decline. Healthy adults ( $n=333$ ,  $M_{age}=70$  years) enrolled in the Australian Imaging, Biomarkers and Lifestyle study underwent A $\beta$  neuroimaging. Neuropsychological assessments were conducted at baseline, 18-, 36- and 54-month follow-ups. A $\beta$  positron emission tomography neuroimaging was used to classify participants as A $\beta^-$  or A $\beta^+$ . Relative to A $\beta^- \epsilon 4^-$ , A $\beta^+ \epsilon 4^+$  individuals showed significantly faster rates of cognitive decline over 54 months across all domains ( $d=0.40-1.22$ ), while A $\beta^+ \epsilon 4^-$  individuals showed significantly faster decline only on verbal episodic memory (EM). There were no differences in rates of cognitive change between A $\beta^- \epsilon 4^-$  and A $\beta^- \epsilon 4^+$  groups. Among A $\beta^+$  individuals,  $\epsilon 4^+/BDNF^{Met}$  participants showed a significantly faster rate of decline on verbal and visual EM, and language over 54 months compared with  $\epsilon 4^-/BDNF^{Val/Val}$  participants ( $d=0.90-1.02$ ). At least two genetic loci affect the rate of A $\beta$ -related cognitive decline. A $\beta^+ \epsilon 4^+/BDNF^{Met}$  individuals can expect to show clinically significant memory impairment after 3 years, whereas A $\beta^+ \epsilon 4^+/BDNF^{Val/Val}$  individuals can expect a similar degree of impairment after 10 years. Little decline over 54 months was observed in the A $\beta^-$  and A $\beta^+ \epsilon 4^-$  groups, irrespective of  $BDNF$  status. These data raise important prognostic issues in managing preclinical AD, and should be considered in designing secondary preventative clinical trials.

## D. HEAT SHOCK PROTEINS (HSPs) AND ALZHEIMER'S DISEASE

**Heat shock proteins (HSPs):** HSPs are a family of proteins that are produced by [cells](#) in response to exposure to stressful conditions. They were first described in relation to [heat shock](#) but are now known to also be expressed during other stresses including exposure to cold,

UV light, and during wound healing or tissue remodeling. Many members of this group perform [chaperone](#) function by stabilizing new proteins to ensure correct folding or by helping to refold proteins that were damaged by the cell stress. Recent studies are showing that HSPs can be neuro-protective in Alzheimer's disease.

[Int J Hyperthermia](#). 2005 Aug;21(5):421-31.

### **Stress proteins in Alzheimer's disease.**

[Smith RC](#)<sup>1</sup>, [Rosen KM](#), [Pola R](#), [Magrané J](#).

Environmental and genetic conditions can cause proteins to misfold or to accumulate abnormally due to impaired clearance. The chaperones which include heat shock proteins, aid survival by preventing protein mis-folding and the formation of cytotoxic protein aggregates. An increasing number of studies point to important roles for molecular chaperones in the biology of neurodegenerative diseases. Heat shock proteins can suppress neurotoxicity in animal models of Parkinson's and polyglutamine diseases, suggesting potential new therapeutic approaches in neurodegenerative disorders associated with abnormal protein folding and toxicity. Recent findings suggest that heat shock proteins can also be neuroprotective in Alzheimer's disease, but this area of research remains largely unexplored. This paper will review the literature related to the role of heat shock proteins in Alzheimer's disease.

[Neuroscience](#). 2000;99(2):317-25.

### **Expression of amyloid precursor protein in human astrocytes in vitro: isoform-specific increases following heat shock.**

[Shepherd CE](#)<sup>1</sup>, [Bowes S](#), [Parkinson D](#), [Cambray-Deakin M](#), [Pearson RC](#).

The beta-amyloid protein deposited in senile plaques and cerebral blood vessels in the Alzheimer's disease brain is derived from the larger transmembrane spanning amyloid precursor protein. The present study investigates the effects of heat shock on the expression and processing of amyloid precursor protein in a normal human fetal astrocytic cell line CC2565 using reverse transcription-polymerase chain reaction, in situ hybridization histochemistry and western blot analysis. Heat shock led to an increase in the messenger RNA encoding Kunitz protease inhibitor isoforms of amyloid precursor protein, which peaked at 4h post-heat shock. This increase was confined to the messenger RNA encoding amyloid precursor protein-751, with a decrease in amyloid precursor protein-770 and no change in amyloid precursor protein-695. This shift in splicing was accompanied by a significant decrease in secreted amyloid precursor protein and an increase in beta-secretase processing within the cell. These findings demonstrate that astrocytes in vitro demonstrate a striking response to heat shock. This is unlikely to be due to a direct action on the promoter region of the gene, since the response is

specific for one splice variant; amyloid precursor protein-751 messenger RNA. This increase in expression is further accompanied by a decrease in secretion of amyloid precursor protein, implying a shift in processing towards an intracellular route, possibly via the actions of the beta-secretase enzyme, which is known to be potentially amyloidogenic. Such a mechanism may contribute to amyloidosis in the intact brain in response to cellular stress, such as head injury. [MolNeurobiol.](#) 2007 Jun;35(3):203-16.

[MolNeurobiol.](#) 2007 Jun;35(3):203-16.

### **Heat shock proteins and amateur chaperones in amyloid-Beta accumulation and clearance in Alzheimer's disease.**

[Wilhelmus MM](#)<sup>1</sup>, [de Waal RM](#), [Verbeek MM](#).

The pathologic lesions of Alzheimer's disease (AD) are characterized by accumulation of protein aggregates consisting of intracellular or extracellular misfolded proteins. The amyloid-beta (Abeta) protein accumulates extracellularly in senile plaques and cerebral amyloid angiopathy, whereas the hyperphosphorylated tau protein accumulates intracellularly as neurofibrillary tangles. "Professional chaperones", such as the heat shock protein family, have a function in the prevention of protein misfolding and subsequent aggregation. "Amateur" chaperones, such as apolipoproteins and heparan sulfate proteoglycans, bind amyloidogenic proteins and may affect their aggregation process. Professional and amateur chaperones not only colocalize with the pathological lesions of AD, but may also be involved in conformational changes of Abeta, and in the clearance of Abeta from the brain via phagocytosis or active transport across the blood-brain barrier. Thus, both professional and amateur chaperones may be involved in the aggregation, accumulation, persistence, and clearance of Abeta and tau and in other Abeta-associated reactions such as inflammation associated with AD lesions, and may, therefore, serve as potential targets for therapeutic intervention.

## Head out/Heat up!

One of the numerous benefits of using the Cocoon IR Wellness Pro System – as opposed to any other sauna-type device—is that your head remains **outside** the heat cabinet during your treatment. Obviously, keeping your head outside the heat cabinet assures a more comfortable and enjoyable treatment. But there are a number of important **scientific reasons** this is more important than you have ever imagined:

1. The Hypothalamus: The hypothalamus is part of the diencephalon which forms part of the cerebral hemispheres and is located in the middle of the brain. The hypothalamus regulates many important functions including the **metabolic rate** and **body temperature**.
2. Brain temperature: Studies have concluded that brain temperature **is the most important stimulus for thermoregulatory responses** which are primarily designed to maintain brain temperature.
3. Prolactin: Exercise in the heat causes the release of prolactin in response to the rise in core body temperature. Increased prolactin release reduces the length of time humans can continue exercising in heat.(3)
4. Thermoregulation: It is believed that there is a coordinated response to exercise involving thermoregulation, neuroendocrine secretion and behavioural adaptations that may originate in the hypothalamus or associated areas of the brain.

Research has shown that keeping the head and face cool reduces brain temperature as a result of cool facial blood returning to the brain (1). This attenuates the normal prolactin response to exercise in the heat without affecting core temperature (2)

A study published in 2008 study investigated the effects of head cooling during endurance cycling on performance and the serotonergic neuroendocrine response to exercise in the heat.(4) The study was conducted to determine whether head cooling has parallel actions on prolactin release, perceived exertion and time to fatigue during submaximal exercise in the heat. Second, the study looked for evidence that keeping the head cool during the exercise acts through direct cooling of the hypothalamus. The study subjects exercised at 75 % VO<sub>2</sub> max to volitional fatigue on a cycle ergometer at an ambient temperature of 29°C. Eight of the nine study subjects were able to exercise longer with head cooling. Head cooling resulted in a **51 % improvement in exercise time** to fatigue and Borg Scale ratings of perceived exertion were significantly lower throughout the exercise period with head cooling. The study further showed that head cooling largely abolished the prolactin response while having no effect on rectal (core) temperature.

The study demonstrated that head cooling led to a considerable improvement in whole body sub-maximal exercise performance in a warm environment. The study further showed that the exercise duration is significantly longer with head cooling. The study authors concluded that the improvement in performance was probably associated with a reduction in central fatigue and changes in prolactin release consistent with the hypothalamus or associated areas being involved in the process. The results suggest there is a potential association between thermoreceptors, the release of stress hormones and central fatigue mechanisms.

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- 1 Cabanac and Caputa 1979, Cabanac, et al.,(1987).
  - 2 Brisson et al.1989, Quirion, et al.,(1990).
  - 3 Marvin, et al. (1997).
  - 4 Ansley, L., Marvin, G., et al., The Effects of Head Cooling on Endurance and Neuroendocrine Responses to Exercise in Warm Conditions, *Physiol. Res.* 57: 863-872, 2008.