# GlycanAge

# Personal report

Test kit ID:

Female-sample-report

Date of sampling:

24 Aug 2025

Date of birth:

06 Apr 1996



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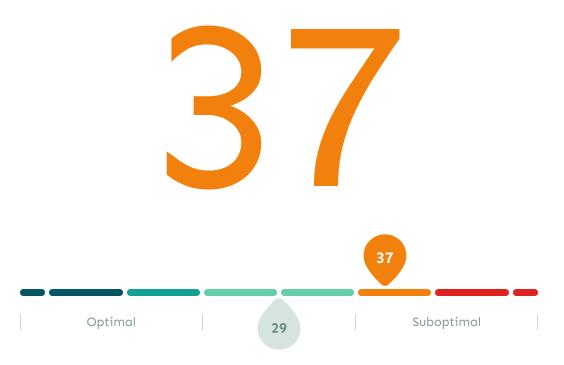
# Key takeaways

# Executive summary

GlycanAge measures **inflammaging**, which indicates the levels of chronic inflammation driven by the immune system as it ages.



# Biological age



This means that your immune system health resembles an average 37 year old individual

## **Result indications**





### **Common causes**

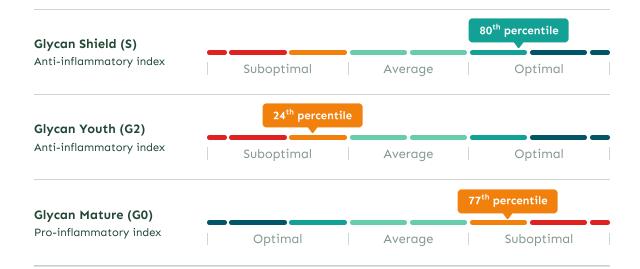


GlycanAge's biological age reflects the health of the immune system and its ability to manage chronic inflammation.

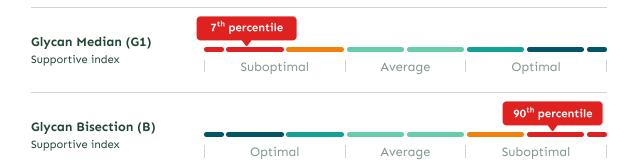
A much higher biological age compared to chronological age is not uncommon and can often be explained by factors such as genetic predisposition, presence of inflammatory diseases, or extreme lifestyle factors.



# Primary glycan indexes



## Supportive glycan indexes



# **Result indications**



#### **Suboptimal Glycan Youth and Glycan Mature**

Two of your key markers are outside the healthy range. Your immune system's youthful, anti-inflammatory capacity is reduced, and it's spending more time in a "switched on" inflammatory state. This combination can speed up immune aging and increase the likelihood of heart, hormone, and immune-related problems in the future. Common drivers include long-term stress, poor sleep, hormone imbalance, and lifestyle habits that don't allow enough recovery.

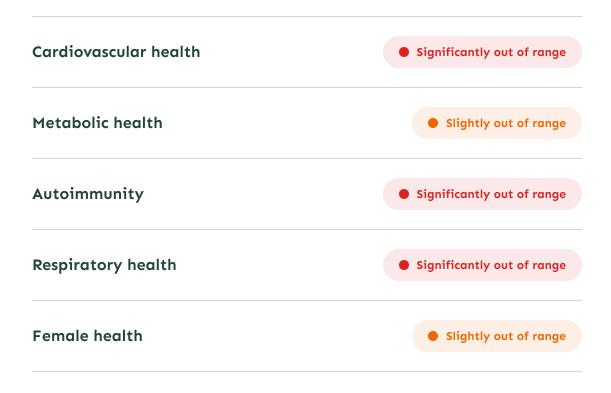


### **Suboptimal Supportive Indexes**

Both your vascular resilience and environmental/autonomic stress markers are outside the healthy range. This can mean your body is under both internal and external pressure — for example, from lifestyle factors, environmental exposures, and reduced recovery ability.



# **Glycan insights**



This page shows how your personal glycan profile relates to key areas of health that are influenced by chronic inflammation.

These insights are based on extensive scientific research from over 300 peer-reviewed papers connecting glycan patterns to different health systems. Full research study is available on **ScienceDirect**.

# **Result indications**



Some health areas are significantly out of range

If a health area is flagged as **Slightly out of range** or **Significantly out of range**, it suggests an increased inflammatory state in that health area.

This is not a diagnosis. Think of it as an early indicator, as glycan changes can reflect processes that occur years before symptoms may appear.

Since your glycan profile can change in response to lifestyle improvements, these insights help you focus on proactive adjustments for your long-term wellbeing.

# **Action steps**

#### Primary focus — based on primary indexes

#### Restore balance and lower inflammation

Focus on steady energy from balanced meals, regular but not excessive exercise, and better recovery habits, including consistent sleep and downtime. Look for ways to reduce stress, such as mindfulness, light movement, or outdoor time.

#### Further testing suggested:

Focus on steady energy from balanced meals, regular but not excessive exercise, and better recovery habits, including consistent sleep and downtime. Look for ways to reduce stress, such as mindfulness, light movement, or outdoor time.

#### Secondary focus — based on secondary indexes

#### Take a whole-body approach to reducing strain

Support your heart and lungs with moderate exercise, protect yourself from environmental triggers (like poor air quality or smoking), and keep a regular daily rhythm to help your body recover.

#### Further testing suggested:

It's worth discussing a full cardiovascular and autoimmunity review with your healthcare provider, including cholesterol, blood pressure checks, lung function testing, and inflammation and autoimmunity markers. A sleep study or heart rate variability tracking may also help identify hidden sources of stress.



## When to retest?



#### Retest in 3-6 months

Your results show significant glycan imbalances, which suggests that chronic inflammation is impacting multiple systems in your body. We strongly advise a retest in 3-6 months.

Your glycans can respond relatively quickly to significant lifestyle changes. This shorter retest interval allows you to closely monitor the effectiveness of your health interventions, helping you make prompt adjustments to your plan and ensuring you're on the most effective path toward improvement.



# Your progress

# Result through time

Tracking biological age and glycan indexes through time provides valuable insights into your **health trajectory**.



# Biological age through time

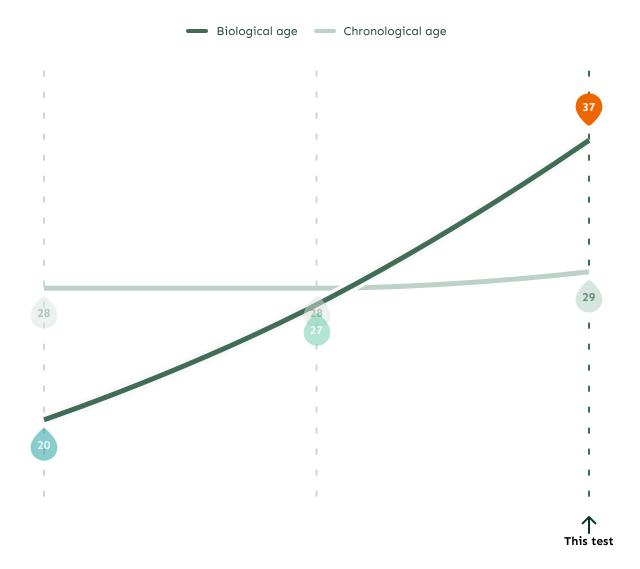
Tracking biological age through time provides valuable insights into your long term health. Monitoring changes in biological age can help to:

- Assess the effectiveness of interventions
- Identify early warning signs of future health decline
- Personalize care plans

### **Understanding result fluctuations**

Chronic inflammation is a dynamic marker of immune health, responsive to both lifestyle and medical interventions.

Since there is no one-size-fits-all approach to health, tracking these fluctuations over time provides a clearer picture of what truly works for an individual—showing whether implemented changes are having the desired effect on inflammation and immune function.



# Glycan indexes through time

Monitoring glycan indexes over time offers a more granular perspective on your health and aging trajectory.

While tracking biological age provides a valuable overall picture, observing changes in specific glycan indexes allows for a deeper understanding of the underlying biological processes and how they are influenced by interventions.

Percentile ranking of index at the time of testing





# **Result details**

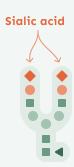
# Glycan indexes breakdown

Glycan structures of similar chemical and functional composition are grouped into 5 different categories called **glycan indexes**.



# Glycan Shield (S)

This index represents glycan structures containing sialic acid (S). It has an anti-inflammatory function. It reduces in abundance with age.



### Optimal results associate with:

- Lower inflammation
- Healthier aging
- Strong immune regulation

### Suboptimal results associate with:

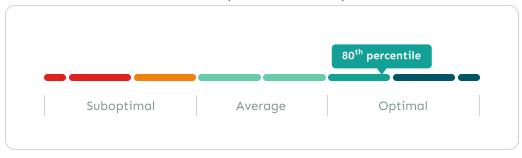
- Unbalanced hormones
- Poor sleep
- Sedentary lifestyle

- Nutritional deficiencies
- Gut and microbiome health

### Compared to people in the same age group and biological sex:



### This result ranks you in the **80**<sup>th</sup> percentile:





# Glycan Youth (G2)

This index represents glycan structures with two terminal galactoses (G2). It has an antiinflammatory function. It reduces in abundance with age.



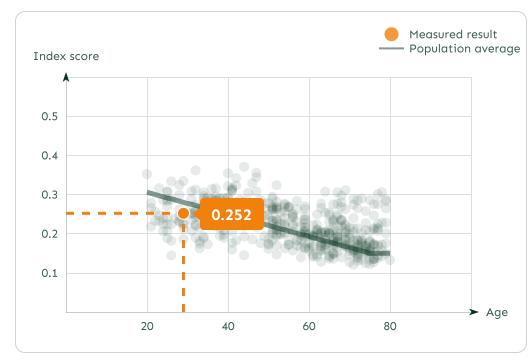
### Optimal results associate with:

- Lower inflammation
- Healthier aging
- Strong immune regulation

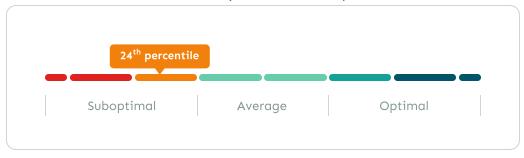
### Suboptimal results associate with:



### Compared to people in the same age group and biological sex:



### This result ranks you in the 24<sup>th</sup> percentile:





# Glycan Mature (G0)

This index represents glycan structures with no terminal galactoses (G0). It has a proinflammatory function. It increases in abundance with age.



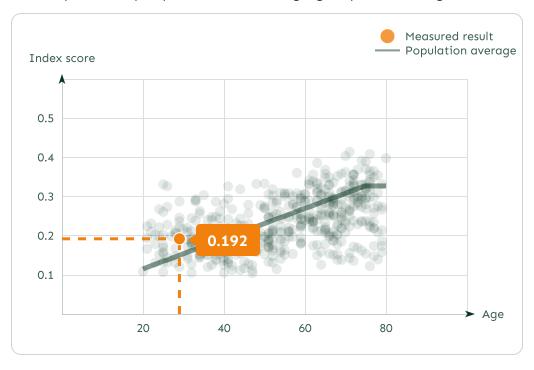
### Optimal results associate with:

- Lower inflammation
- Healthier aging
- Strong immune regulation

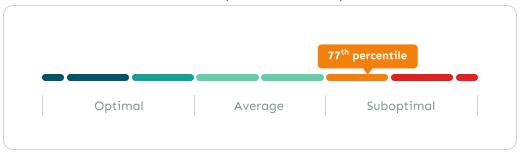
### Suboptimal results associate with:



#### Compared to people in the same age group and biological sex:



### This result ranks you in the 77<sup>th</sup> percentile:





# Glycan Median (G1)

This index represents glycan structures with one terminal galactose (G1). As a supportive index, it can help narrow down associations with specific disease types, genetic traits, and/or some lifestyle habits.



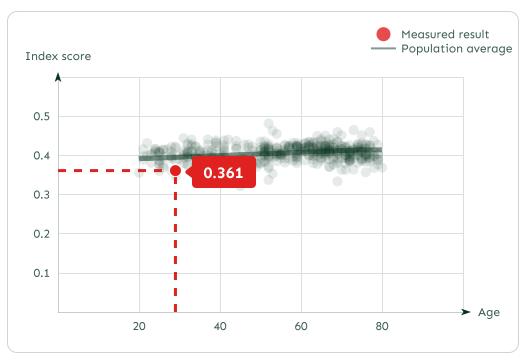
### Optimal results associate with:

- Balanced immune adaptation
- Recent lifestyle changes
- Cardioprotective effects

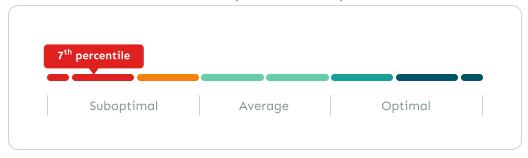
### Suboptimal results associate with:

- Lower immune resilience
- Cardiovascular risk
- Disrupted immune adaptation

### Compared to people in the same age group and biological sex:



#### This result ranks you in the **7**<sup>th</sup> percentile:



This page is a new feature in the report. See <u>changelog</u> for more info.



# Glycan Bisection (B)

This index represents glycan structures with a bisecting (B) GlcNAc modification. As a supportive index, it can help narrow down associations with specific disease types, genetic traits, and/or some lifestyle habits.



### Optimal results associate with:

Strong immune regulation

Healthy habits

### Suboptimal results associate with:

Impaired adaptive response

Reduced resilience

Smoking

Chronic stress

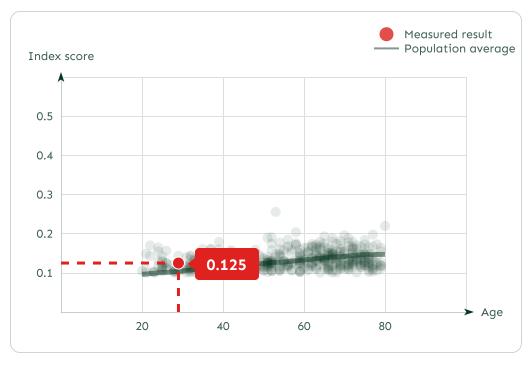
Toxin exposure

Alcohol intake

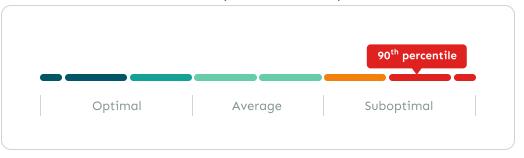
Poor sleep

Blood pressure disregulation

### Compared to people in the same age group and biological sex:



### This result ranks you in the 90<sup>th</sup> percentile:





# **Additional impacts**

# Influencing factors

**Lifestyle, life stages**, as well as **pharmacological interventions** can have a significant impact on chronic inflammation, which will likely reflect on GlycanAge results.



Dietary habits shape inflammation levels and affect the GlycanAge score. While short-term lapses in diet, such as an occasional indulgence, won't impact the GlycanAge score, long term changes in diet will most likely affect the results.

There is no one-size-fits-all diet, as each person's metabolism is unique and finding the optimal diet for an individual is a difficult task. This makes GlycanAge a valuable tool that can help you understand whether your patient's diet is good for them or if it fuels inflammation.

Our research found that in overweight individuals, only caloric restriction has an overall anti-inflammatory effect in most people whereas other types of diets show different effects depending on a individual.





#### Related research papers

# Effects of low-calorie and different weight-maintenance diets on IgG glycome composition

The study investigated the effects of different diets on IgG glycans, analyzing 1,850 samples from the Diogenes study, one of the largest dietary intervention studies. A total of 938 participants who were overweight underwent an 8-week low-calorie diet (800 kcal/day), followed by one of the weight maintenance diets for 6 months.

- Low protein / low glycemic index
- Low protein / high glycemic index
- High protein / low glycemic index
- High protein / high glycemic index

Only caloric restriction (CR) resulting in weight loss showed anti-inflammatory effects. After 8 weeks of CR, a decrease was observed in the pro-inflammatory Glycan Mature index and an increase in the anti-inflammatory Glycan Shield index. These changes returned to baseline after some of the weight was gained back on maintenance diets.

While no statistically significant changes in IgG glycans were observed on the maintenance diets, individual responses varied—some participants showed improvement, while others experienced worsening results. This variability underscores the importance of a personalized approach to nutrition showing there is no one-size-fits-all diet.

### **Stress**

When the body experiences stress, it activates the fight-or-flight response, which is beneficial in short-term situations. However, chronic stress disrupts the hormonal balance, keeping hormones like adrenaline and cortisol high, which leads to disruption of the immune system and fuels inflammation.

Therefore, individuals who are under a lot of stress can have higher GlycanAge scores. Both individuals under constant stress, such as work-related pressure, and those who encounter sudden, intense stress, like major life events, may experience an increase in their GlycanAge due to the body's sustained inflammatory response.





#### Related research papers

N-glycosylation profiling of plasma provides evidence for accelerated physiological aging in post-traumatic stress disorder

The study aimed to explore whether traumatic stress accelerates the aging process by analyzing glycan profiles in individuals experiencing varying levels of stress. A total of 32 participants were included: 13 individuals with post-traumatic stress disorder (PTSD), 9 trauma-exposed individuals without PTSD, and 10 low-stress control subjects. The researchers used the GlycoAge test, a biomarker for physiological aging, and found that, on average, individuals with PTSD and those exposed to trauma showed signs of accelerated aging by 15 years compared to the low-stress controls./day), followed by one of the weight maintenance diets for 6 months.

# Insufficient sleep

Insufficient sleep has been shown to raise inflammatory markers in the body. Both acute sleep deprivation (e.g., being awake for 24 hours) and chronic insufficient sleep can increase inflammation.

While short-term sleep deprivation does not affect the GlycanAge score, long-term insufficient sleep can cause elevated GlycanAge score.

Additionally, individuals with sleep disorders such as sleep apnea, which disrupts normal breathing during sleep, may also have higher scores due to the inflammatory nature of this condition. Our research indicates that people with severe obstructive sleep apnea (OSA) are, on average, 6.9 years older biologically than their chronological age.





#### Related research papers

Not-So-Sweet Dreams: Plasma and IgG N-Glycome in the Severe Form of the Obstructive Sleep Apnea

The aim of the study was to explore whether IgG glycans can be used as biomarkers for severe obstructive sleep apnea (OSA). IgG and total plasma glycans were analyzed in 70 subjects with severe OSA and 23 controls. Significant changes were observed in both IgG and total plasma glycans. Furthermore, patients with severe OSA exhibited accelerated biological aging, with GlycanAge score on average being 6.9 years higher than their chronological age. This study suggests that both IgG and total plasma glycans might be considered biomarkers for severe OSA./day), followed by one of the weight maintenance diets for 6 months.

# **Exercise**

Regular physical activity and exercise have positive effects on biological age and glycan indexes.

However, individuals who engage in exercise after a long period of inactivity and sedentary lifestyle may initially experience an increase in chronic inflammation and their biological age, especially if they are overweight.

Exercise is also crucial for cardiovascular health and studies in women show that exercise has positive effects on a specific glycan that has a cardio protective role.





#### Related research papers

<u>Physical Exercise Induces Significant Changes in Immunoglobulin G N-Glycan</u>
<u>Composition in a Previously Inactive, Overweight Population</u>

The study investigated the impact of regular exercise on IgG glycans in previously inactive, middle-aged, overweight population. 397 participants were subjected to one of the following exercise programs for 12 weeks:

- circular exercise program
- cardio exercise program
- Nordic walking program

After completing the program, the participants showed an increase in some proinflammatory glycans, which was somewhat expected as they were previously inactive.

The main result of the study was an increase in a specific glycan structure (GP9) which is reported to have a protective role in cardiovascular health in women.

# Overexercising

Although exercise and regular physical activity are crucial for good health, overexercising can have negative impacts on levels of inflammation.

The acute inflammation caused by a workout is beneficial. However, constant overexercise without proper recovery period can cause an increase in low-grade systemic inflammation and may increase the GlycanAge score.

Professional athletes and individuals with extreme exercise regimens generally have a higher GlycanAge score compared to those who engage in moderate and balanced exercise.





#### Related research papers

Regular moderate physical exercise decreases Glycan Age index of biological age and reduces inflammatory potential of Immunoglobulin G

The study included 276 healthy participants divided into 4 groups based on their activity level:

- inactive group
- newly involved recreational group
- regularly moderate active group
- professionally competing athlete group

On average, those who exercise regularly had the lowest GlycanAge score when compared to other groups. It was found that those who exercises regularly had on average a lower GlycanAge score by 7.4 years when compared to inactive individuals (around 10 years for women and 6 for men). Professional athletes showed an increased GlycanAge score by 7.6 years on average compared to those who exercise regularly, however, this trend was observed in women only.

#### **Additional notes**

Some forms of intense exercise, such as repeated sprint training (RST), show positive effects on glycans and lead to a reduction in biological age.

# Weight loss

Excess body weight significantly influences IgG glycans and is associated with a higher GlycanAge score and poor index scores.

Weight loss, whether through dieting, exercise, or bariatric surgery, generally leads to a reduction in the GlycanAge score and improvements in nearly all indexes.

However, extreme weight loss can temporarily increase the GlycanAge score. Upon fat tissue reduction, inflammatory molecules stored in the fat are released into the bloodstream, causing increased inflammation.











#### Related research papers

#### Extensive weight loss reduces glycan age by altering IgG N-glycosylation

Individuals scheduled for bariatric surgery (n=37) were subjected to 3 weeks of low-calorie diet (900 kcal/daily). In the short period while under caloric restriction, an improvement in the Glycan Bisection index was observed, indicating a reduced proinflammatory potential of IgG glycans.

Following the bariatric surgery, additional improvements such as a decrease in the pro-inflammatory Glycan Mature index and an increase in anti-inflammatory indexes Glycan Youth and Glycan Mature were observed.

The results were further validated on 1680 individuals from the TwinsUK cohort followed for 20 years where it was observed that reduction of BMI through weight loss was associated with a reduced GlycanAge score and improvements in the Glycan Mature and Glycan Youth indexes.

#### **Additional notes**

In another <u>study</u>, we investigated the effects of different diet types on IgG glycans and found that caloric restriction, rather than a specific type of diet, is the main driver of positive changes.

# **Supplements**

Supplements can affect the GlycanAge score both positively and negatively, as individuals have different responses to them.

Our studies looking at the effects of omega-3s and NAD+ precursor supplements, show positive effects on the GlycanAge score and indexes.

Experimentally we know various other supplements affect the GlycanAge score, especially if the supplements target common deficiencies (e.g. vitamin D) or have anti-inflammatory properties.

However, not all supplements are suitable for everyone and GlycanAge can help you understand how your patient is responding to them.





#### Related research papers

The effect of n-3 polyunsaturated fatty acids-enriched hen eggs consumption on IgG and total plasma protein N-glycosylation in healthy individuals and cardiovascular patients

The study investigated the effects of omega-3 PUFAs-enriched hen eggs consumption on IgG glycans and other inflammatory biomarkers in healthy people and in cardiovascular (CV) patients. In healthy individuals who consumed omega-3 enriched eggs, we saw a decrease in the Glycan Bisection index and in those consuming normal eggs we saw an increase in the Glycan Mature index. In CV patients, we saw a switch towards a less-inflammatory profile of the total blood plasma glycans.

#### The use of a systems approach to increase NAD+ in human participants

This double-blinded, placebo-controlled crossover trial investigated the efficacy of the NAD+ supplement Nuchido TIME+. Healthy participants (n=26) aged 21-72 were randomized to receive either the supplement or a placebo for 28 days, followed by a one-week washout period, after which the groups switched treatments. Participants taking the NAD+ supplement experienced an average decrease in their GlycanAge score by 1.26 years after only 28 days, a change not observed in those receiving the placebo.

## Metformin

Metformin is a prescription drug commonly used for treating type 2 diabetes and insulin resistance. Due to its supposed benefits, it is also being used in nondiabetics.

The effects of metformin are still being researched, especially for non-diabetics. Our studies show that metformin generally does not significantly influence GlycanAge in non-diabetics. However, some individuals do experience changes in their GlycanAge scores while on metformin, reflecting their unique response to the treatment.





#### Related research papers

<u>Effects of testosterone and metformin on the GlycanAge index of biological age</u> and the composition of the <u>IgG glycome</u>

This clinical trial investigated the effects of metformin and testosterone replacement therapy (TRT) in 82 male participants dealing with obesity and low testosterone levels. They were randomized into receiving:

- metformin
- TRT
- metformin+TRT
- placebo

Samples were taken at 3 timepoints - before starting the treatment, at the 6-month mark, and again one year into the treatment. Significant changes in IgG glycans were observed only in the groups receiving TRT, whether alone or combined with metformin, indicating that these changes were attributed to TRT rather than metformin. In the metformin group, there was no consistent trend in GlycanAge scores - some individuals experienced a minor increase, while others experienced a minor decrease. The exception was one participant who showed a significant decrease in their GlycanAge score after taking metformin.

#### **Additional notes**

Preliminary data shows that metformin has some positive effects on IgG glycans in diabetics.

# **Irregular cycles**

During a woman's menstrual cycle, hormones fluctuate cyclically, and IgG glycans also change in a cyclic pattern. However, these changes are typically not significant enough to affect the overall GlycanAge score, so the menstrual cycle phase generally does not influence the results.

However, for women with irregular menstrual cycles or conditions like polycystic ovary syndrome (PCOS) or endometriosis, which are characterized by hormonal imbalances and increased inflammation, the GlycanAge score may be higher.





#### Related research papers

# <u>Periodic Changes in the N-Glycosylation of Immunoglobulin G During the Menstrual Cycle</u>

The study examined longitudinal changes in IgG glycans during the menstrual cycle in a cohort of healthy premenopausal women with regular cycles (n=70). The women were sampled at 12 time points during their cycle—every 7 days for 3 months. Although the average variation in Glycan indexes was only up to 1.1%, the changes exhibited a cyclic pattern.

The folicular phase was characterized by the highest abundance of Glycan Mature, Glycan Median, and Glycan Bisection indexes, which are known to have proinflammatory properties. After ovulation, we saw an increase in anti-inflammatory Glycan Youth and Glycan Shield indexes.

These variations were associated with female sex hormones and menstrual cycle phases, however, the changes in Glycan indexes didn't overlap with the highest concentrations of sex hormones but appeared as menstrual cycle phase-specific events. Despite the observed changes in IgG glycans, they weren't significant enough to affect the overall GlycanAge score.

#### **Additional notes**

If the patient has irregular cycles due to entering peri-menopause, you can refer to the <u>perimenopause</u> page for more information.

# **Pregnancy**

During pregnancy, estrogen levels increase significantly, with a specific form of estrogen being predominantly produced by the placenta (estradiol E2).

Estrogen has known anti-inflammatory properties, similar to the effects seen with IgG glycans. During pregnancy, women can experience a reduction in their GlycanAge score, along with improvements in anti-inflammatory indexes (Glycan Youth, Glycan Shield).









#### Related research papers

Immunoglobulin G galactosylation and sialylation are associated with pregnancy-induced improvement of rheumatoid arthritis and the postpartum flare: results from a large prospective cohort study

We investigated changes in IgG glycans in Caucasian women diagnosed with rheumatoid arthritis (RA) from pre-pregnancy until six months postpartum. The study included 148 RA patients and 32 healthy controls. We observed an increase in the anti-inflammatory Glycan Youth and Glycan Shield indexes from preconception until the end of pregnancy, which was associated with remission of RA symptoms during pregnancy. After pregnancy, we observed a significant decrease in both Glycan Youth and Glycan Shield indexes, reaching their lowest levels at six months postpartum. This decrease was associated with a flare-up in RA severity.

#### **Additional notes**

Pregnancy has been observed to improve chronic conditions like rheumatoid arthritis, which often flare up again postpartum. These changes are likely due to the pregnancy-induced increase in estrogen and are also reflected in glycan levels and indexes.

# **Post-pregnancy**

After giving birth, woman's body undergoes significant changes as hormone levels gradually return to their pre-pregnancy state.

The marked increase in estrogen observed during pregnancy diminishes, often leading to an increase in the GlycanAge score. This is often reflected by a reduction in anti-inflammatory indexes (Glycan Shield, Glycan Youth).

- A Biological age
  Chronic inflammation
- Glycan Shield
  Anti-inflammatory
- Glycan Youth
  Anti-inflammatory



#### Related research papers

Immunoglobulin G galactosylation and sialylation are associated with pregnancy-induced improvement of rheumatoid arthritis and the postpartum flare: results from a large prospective cohort study

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# Perimenopause

Perimenopause is a phase preceding menopause in which estrogen levels start to drop.

Before perimenopause, women exhibit a similar aging pace to men. However, upon entering perimenopause, their GlycanAge score rises abruptly, reflecting the increase in inflammation due to a large drop in estrogen levels.

Perimenopause is predominantly characterized by an increase in the pro-inflammatory Glycan Mature index and a decrease in the anti-inflammatory Glycan Youth index. Studies also observe an increase in Glycan Bisection index.





Glycan Shield
Anti-inflammatory

Glycan Bisection
Supportive index





#### Related research papers

# <u>Immunoglobulin G glycome composition in transition from premenopause to postmenopause</u>

The study analyzed the IgG glycome in 5080 samples from 1940 females multiple times during their transition from pre-menopause to menopause. The most prominent changes associated with entering menopause were an increase in the Glycan Mature and Glycan Bisection indexes and a subsequent decrease in Glycan Youth and Glycan Shield. These changes are known to occur with aging in general, however, they were more pronounced in peri-menopausal women compared to women of other age groups and to men.

#### Additional notes

During their transition from pre- to peri-menopause, women can experience an increase in their GlycanAge score by over a decade.

# Hormone replacement therapy

Hormone replacement therapy (HRT) is often used in managing symptoms of peri- and menopausal women to restore hormonal balance, especially estrogen.

Estrogen is a known modulator of IgG glycans that has anti-inflammatory properties.

Women who undergo HRT often experience a reduction in their GlycanAge score and a reduction in the pro-inflammatory Glycan Mature index.







#### Related research papers

#### Effects of estradiol on biological age measured using the glycan age index

Postmenopausal women (n=58) with low estrogen levels were randomized to receive estrogen, raloxifene (medication used to relieve some symptoms of menopause) or placebo. Those on estrogen and raloxifene showed positive changes such as a reduction in the pro-inflammatory Glycan Mature index.

To confirm the effects of estrogen even further, pre-menopausal healthy women (n=21) were treated with leuprolide, which lowered estrogen production, mimicking symptoms of menopause. Some women received transdermal estrogen and others were on placebo.

The placebo group experienced an increase in the pro-inflammatory Glycan Mature index and showed an average increase in their GlycanAge score by 9,1 years. This effect was prevented by transdermal estrogen therapy. After recovery, GlycanAge scores of women returned to baseline.

#### **Additional notes**

HRT in women isn't just about optimizing estrogen - it's about finding the right balance between all female sex hormones, including progesterone.

# **Anti-Estrogen therapy**

Anti-estrogen therapies are used to treat hormone receptor-positive (HR-positive) breast cancer, which accounts for 70-80% of all cases. These therapies, which work through different mechanisms, all block estrogen's effects on cancer cells, slowing or stopping tumor growth.

Estrogen has an anti-inflammatory effect on IgG glycans, so anti-estrogen therapies can shift IgG glycans toward a more pro-inflammatory profile, potentially increasing biological age. However, our studies show that different anti-estrogens affect IgG glycans in distinct ways.

Anastrozole, an aromatase inhibitor, blocks the final step in estrogen production, while tamoxifen, an estrogen antagonist, competitively binds to estrogen receptors. Women on anastrozole show a decrease in the anti-inflammatory Glycan Shield index, whereas women on tamoxifen show an increase.

#### **Anastrozole**



#### **Tamoxifen**





#### Related research papers

<u>Anastrozole and Tamoxifen Impact on IgG Glycome Composition Dynamics in</u>
Luminal A and Luminal B Breast Cancers

The study explored the impact of anti-estrogen therapies on IgG glycans in 40 women diagnosed with Luminal A and B subtypes of breast cancer. It focused on two therapies: anastrozole, an aromatase inhibitor, and tamoxifen, a selective estrogen receptor modulator (SERM).

The results showed distinct effects between the two therapies. Anastrozole was associated with a shift toward a more pro-inflammatory profile, with increases in Glycan Mature and Glycan Bisection indexes, and decreases in Glycan Youth, Glycan Median, and Glycan Shield. In contrast, tamoxifen showed the opposite effect across most indexes, except for Glycan Bisection and Glycan Youth. The most notable difference was in the Glycan Shield index, which decreased with anastrozole and increased with tamoxifen.



# **Appendix**

# Science behind



# GlycanAge 101 Part 1/3

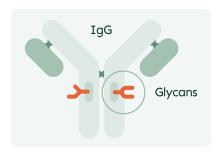
### What is GlycanAge?

GlycanAge is a biological age test. It assesses the health of the immune system by measuring levels of chronic inflammation.

## Is GlycanAge a diagnostic tool?

GlycanAge can be categorized as longevity diagnostics. It provides valuable insights into potential health risks and areas for intervention. It isn't a traditional diagnostic tool, since aging isn't considered a disease.

### What does GlycanAge analyze?



GlycanAge analyzes glycans (complex sugars) attached to immunoglobulin G (IgG) antibodies. These glycans regulate inflammatory responses in the body.

### How is biological age calculated?

GlycanAge calculates biological age by combining glycans most correlated with aging (primary glycan indexes) into a score and comparing it to a baseline of healthy individuals aged 20-80.

### What are the benefits of using GlycanAge?

Chronic inflammation is a key driver of disease development, influenced by genetics, environment, and lifestyle. GlycanAge provides a deeper understanding of inflammation at a molecular level, reflecting biological changes that precede disease and serving as a predictive biomarker for future health outcomes.

GlycanAge is a modifiable biomarker responsive to both lifestyle and medical interventions. It reflects individual responses to treatments in as little as 3 months, and can serve as a tool to measure the effectiveness of introduced changes.

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# GlycanAge 101 Part 2/3

### What are glycan indexes?

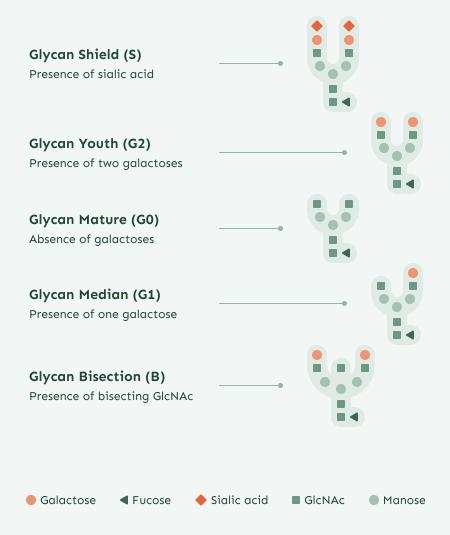
GlycanAge analyzes 29 different glycan structures gathered from the blood sample. Glycan structures of similar chemical and functional composition are grouped into 5 different categories called glycan indexes.

**Primary indexes** have a clear pro- or anti-inflammatory function, and are used to calculate the biological age of a person. Primary indexes are:

- Glycan Shield (anti-inflammatory),
- Glycan Youth (anti-inflammatory),
- Glycan Mature (pro-inflammatory).

**Supportive indexes** can help narrow down associations with specific disease types, genetic traits, and/or some lifestyle habits. Supportive indexes are:

- Glycan Median
- Glycan Bisection





# GlycanAge 101 Part 3/3

### What are glycan indexes?

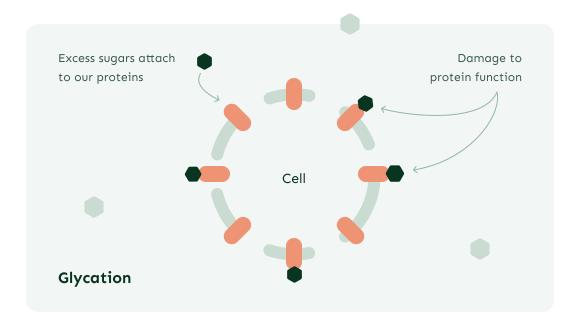
No. GlycanAge measures glycosylation, a controlled process which is fundamentally different from the random, damaging process of glycation.

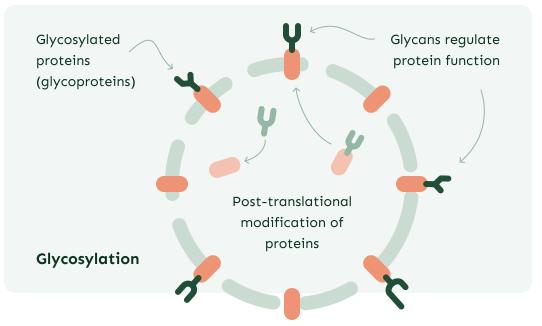
### **Glycation** (what we don't measure):

- A random, uncontrolled chemical reaction.
- Damages proteins due to excess sugar exposure.
- Contributes to aging and is linked to high blood sugar.

### **Glycosylation** (what we do measure):

- A precise, controlled, and vital biological process.
- Regulates protein function, telling them what to do.
- Glycosylation patterns in IgG provide insight into chronic inflammation and biological age.







## **Technical information**

### **CLIA-certified laboratory**

Our laboratory is CLIA-certified, ensuring adherence to the highest standards of quality and accuracy in laboratory testing.



- Plasma is used as the starting material for the isolation of Immunoglobulin G (IgG), which is then glycoprofiled. IgG is isolated from the complex mixture of plasma proteins using affinity chromatography to specifically analyze its glycosylation. The concentration of the isolated IgG is determined using a UV-Vis method on the Nanodrop 8000. To assess chronic inflammation markers, the glycoprofiling of isolated IgG is performed.
- 2. For the analysis of N-glycans, samples undergo deglycosylation, where N-glycans are released from glycoproteins using the PNGase F enzyme. Fluorescent labeling of N-glycans is then performed, preparing the N-glycans for subsequent separation and quantification.

- 3. Capillary Gel Electrophoresis with Laser-Induced Fluorescence (CGE-LIF) is employed for glycoprofiling. This method enables high-throughput analysis with superior sensitivity. The glycoprofiles are generated using an ABI 3500 DNA sequencer, where the complex mixture of IgG glycans is separated into 27 glycan peaks.
- 4. Glycans under each peak are relatively quantified and compared to a reference population using a proprietary algorithm. This approach ensures accurate and reproducible quantification of glycan species, providing insights into disease-associated glycan structures and their relevance to inflammatory processes.

# Research papers

### **Biological** age

Glycans Are a Novel Biomarker of Chronological and Biological Ages

Immunoglobulin G glycans - Biomarkers and molecular effectors of aging

Heritability of the glycan clock of biological age

Immunoglobulin G glycosylation in aging and diseases

IgG glycans in health and disease: Prediction, intervention, prognosis, and therapy

### **Influencing factors**

Immunoglobulin G galactosylation and sialylation are associated with pregnancy-induced improvement of rheumatoid arthritis and the postpartum flare: results from a large prospective cohort study

Periodic Changes in the N-Glycosylation of Immunoglobulin G During the Menstrual Cycle

Immunoglobulin G glycome composition in transition from premenopause to postmenopause

Effects of estradiol on biological age measured using the glycan age index

Estrogens regulate glycosylation of IgG in women and men

The effect of n-3 polyunsaturated fatty acids-enriched hen eggs consumption on IgG and total plasma protein N-qlycosylation in healthy individuals and cardiovascular patients

The use of a systems approach to increase NAD+ in human participants

Extensive weight loss reduces glycan age by altering IgG N-glycosylation

Effects of testosterone and metformin on the GlycanAge index of biological age and the composition of the IgG glycome

Regular moderate physical exercise decreases Glycan Age index of biological age and reduces inflammatory potential of Immunoglobulin G

N-glycosylation profiling of plasma provides evidence for accelerated physiological aging in post-traumatic stress disorder

Not-So-Sweet Dreams: Plasma and IgG N-Glycome in the Severe Form of the Obstructive Sleep Apnea

Intense Physical Exercise Induces an Anti-inflammatory Change in IgG N-Glycosylation Profile

<u>Physical Exercise Induces Significant Changes in Immunoglobulin G N-Glycan Composition in a Previously Inactive, Overweight Population</u>

#### Diseases

N-glycosylation of immunoglobulin G predicts incident hypertension

The Association Between Glycosylation of Immunoglobulin G and Hypertension: A Multiple Ethnic Cross-Sectional Study

Immunoglobulin G N-Glycosylation Signatures in Incident Type 2 Diabetes and Cardiovascular Disease

<u>Glycosylation Profile of Immunoglobulin G Is Cross-Sectionally Associated With Cardiovascular Disease Risk</u>
Score and Subclinical Atherosclerosis in Two Independent Cohorts

IgG N-Glycosylation Is Altered in Coronary Artery Disease

IgG glycan patterns are associated with type 2 diabetes in independent European populations

The changes of immunoglobulin G N-glycosylation in blood lipids and dyslipidaemia

Association between galactosylation of immunoglobulin G and improvement of rheumatoid arthritis during pregnancy is independent of sialylation

IgG Fc galactosylation predicts response to methotrexate in early rheumatoid arthritis

<u>Low galactosylation of IgG associates with higher risk for future diagnosis of rheumatoid arthritis during 10</u> years of follow-up

Inflammatory bowel disease associates with proinflammatory potential of the immunoglobulin G glycome

Glycosylation of Immunoglobulin G Associates With Clinical Features of Inflammatory Bowel Diseases

A unique serum IgG glycosylation signature predicts development of Crohn's disease and is associated with pathogenic antibodies to mannose glycan

<u>Association of Systemic Lupus Erythematosus With Decreased Immunosuppressive Potential of the IgG</u> Glycome

N-glycosylation patterns of plasma proteins and immunoglobulin G in chronic obstructive pulmonary disease

Estrogens regulate glycosylation of IgG in women and men

Immunoglobulin G glycome composition in transition from premenopause to postmenopause