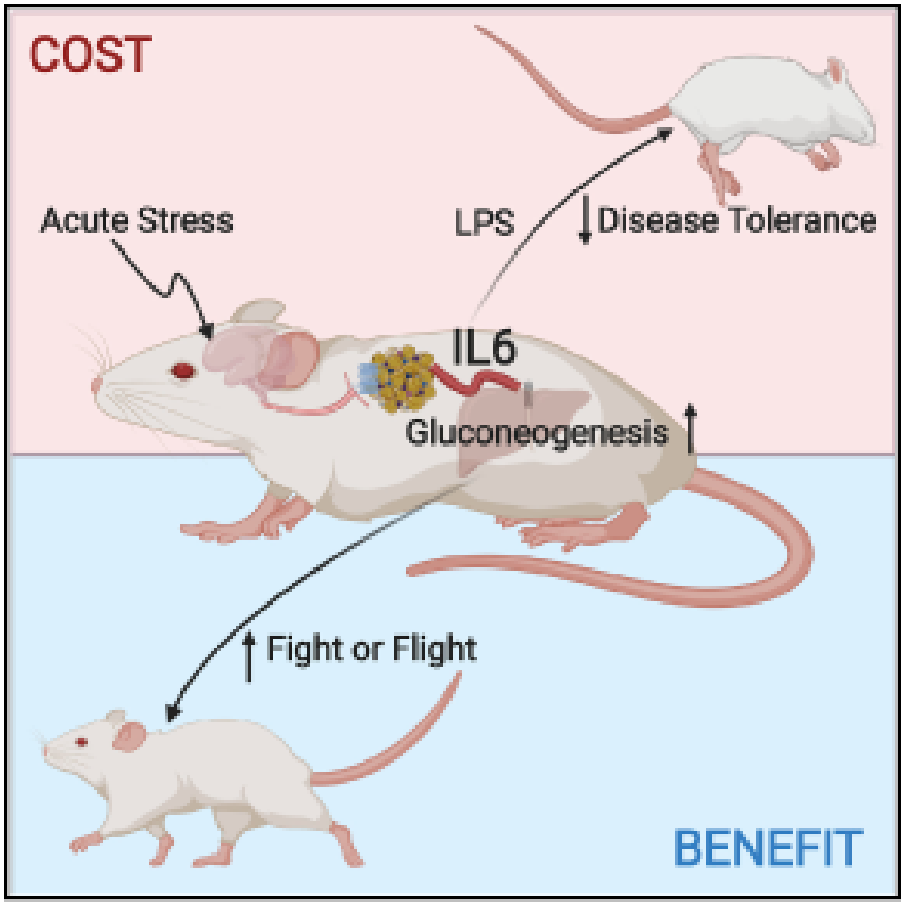
Cell

Origin and Function of Stress-Induced IL-6 in Murine Models

Graphical Abstract



Authors

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In Brief

During acute psychological stress, brown adipocytes initiate a chain of events mediated by adrenergic signaling and IL-6 release that metabolically fuels "fight or flight" adaptive responses but at the same time comes at an inflammatory cost.

Highlights

- IL-6 is the dominant endocrine cytokine induced by acute stress in mice
- Stress-inducible IL-6 is produced in brown adipocytes via ADRB3 signaling
- IL-6 is required for stress hyperglycemia and adaptive "figh or flight" responses
- Stress-induced IL-6 decreases tolerance to a subsequent inflammatory challenge

SUMMARY

Acute psychological stress has long been known to decrease host fitness to inflammation in a wide variety of diseases, but how this occurs is incompletely understood.

Using mouse models, we show that interleukin-6 (IL-6) is the dominant cytokine inducible upon acute stress alone.

Stress-inducible IL-6 is produced from brown adipocytes in a beta-3-adrenergic-receptor-dependent fashion.

During stress, endocrine IL-6 is the required instructive signal for mediating hyperglycemia through hepatic gluconeogenesis, which is necessary for anticipating and fueling ‘‘fight or flight’’ responses.

This adaptation comes at the cost of enhancing mortality to a subsequent inflammatory challenge.

These findings provide a mechanistic understanding of the ontogeny and adaptive purpose of IL-6 as a bona fide stress hormone coordinating systemic immunometabolic reprogramming. This brain-brown fat-liver axis might provide new insights into brown adipose tissue as a stress-responsive endocrine organ and mechanistic insight into targeting this axis in the treatment of inflammatory and neuropsychiatric diseases.

Question:

How does psychological stress, which leads to the production of immunosuppressive mediators such as cortisol and catecholamines, decrease host fitness to inflammation?

Studies dating back to 1990 have shown that psychological stress increases circulating levels of interleukin-6 (IL-6) in humans and laboratory animals.

The role that IL-6 plays in the acute stress response, also referred to as the ‘‘fight or flight’’ response, is unclear.

The idea that stress itself induces endocrine mediators like IL-6, which is traditionally associated with inflammation, has since been supported by the detection of increased circulating cytokines in depression and anxiety

Here, we report that:

1) commonly utilized models of acute stress in mice induce endocrine IL-6

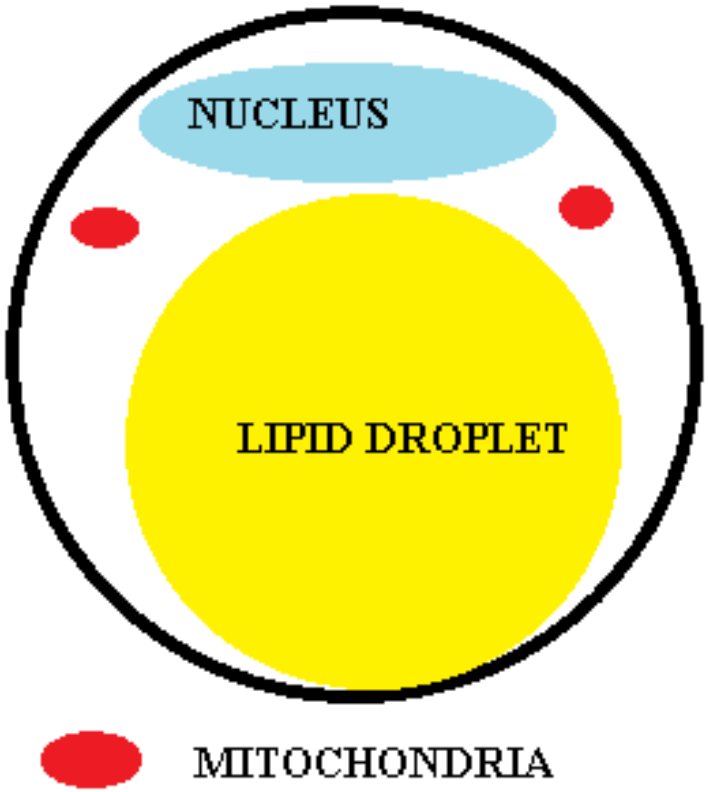
2) Stress-induced IL-6 requires consciousness and beta-3-adrenergic-receptor signaling in brown adipocytes

3) IL-6 is required for stress hyperglycemia, a metabolic adaptation that enables the ‘‘fight or flight’’ response, via hepatic gluconeogenesis.

4) The cost of stress-induced IL-6 is that it decreases host fitness to a subsequent inflammatory challenge.

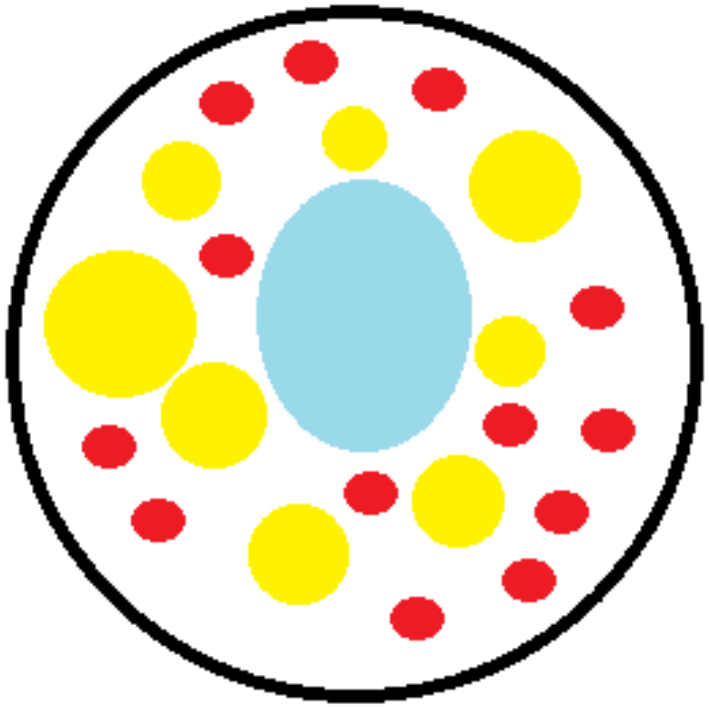
WHITE FAT

ENERGY STORAGE



BROWN FAT

HEAT GENERATION



Distinction between brown and white adipose tissue

parameters	Brown fat	White fat
Essential function	Thermogenesis - energy expenditure	Energy storage
Anatomical distribution	Restricted-but dispersed BAT fat cells exist in fat deposits	Extensive - cell size heterogeneity
Vascularization	Extensive	Relatively sparse
Sympathetic innervation	Extensive (vasculature but also adipocytes)	Relatively sparse alongside blood vessels
Adipocyte precursors	Express UCP (33,000 kDa protein of mitochondria)	Do not express UCP
Fat droplet	Multilocular	Unilocular
Mitochondria	Large number with a well-developed cristae structure Regulated uncoupling	Restricted number with few cristae

Link between stress and inflammation: CONTRADDICTION

Most **chronic sterile inflammatory diseases** are known to **"flare" after acute stress**, contributing significantly to morbidity and mortality.

Indeed, **psychosocial stress worsens most inflammatory diseases, including allergic diseases, autoimmune diseases, and cancers**

However the well-studied mediators of stress physiology, glucocorticoids, and catecholamines, are primarily thought to be immunosuppressive

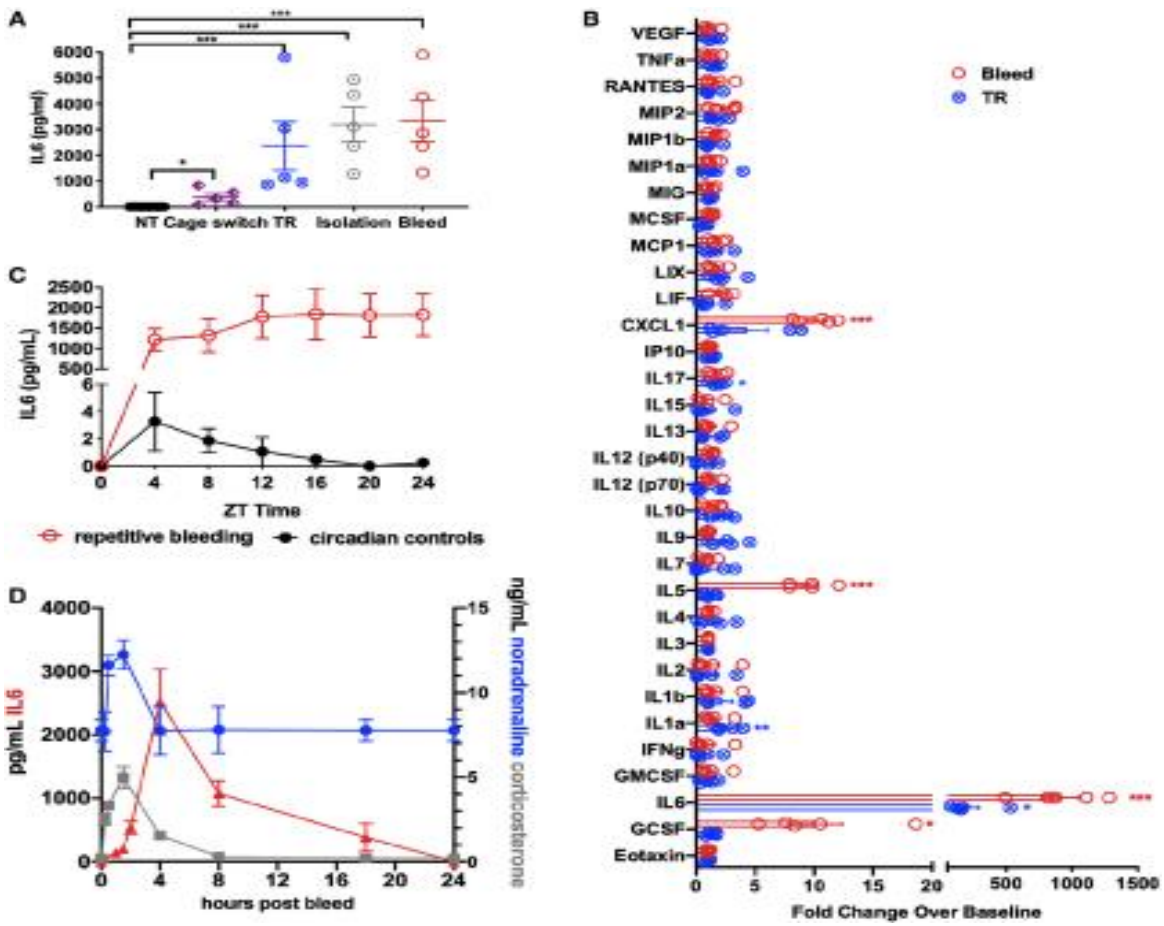
a paradox that many have tried to resolve for over 30 years

Fig1 Acute stress induces endocrine IL-6

Standard laboratory models of acute stress— including tube restraint, cage switching, and social isolation- induced high levels of circulating IL-6 a single, conscious, retro-orbital bleed induced IL6.

Among 32 immunological markers IL-6 was the most greatly induced cytokine and common to two different stress models (Figure 1B)

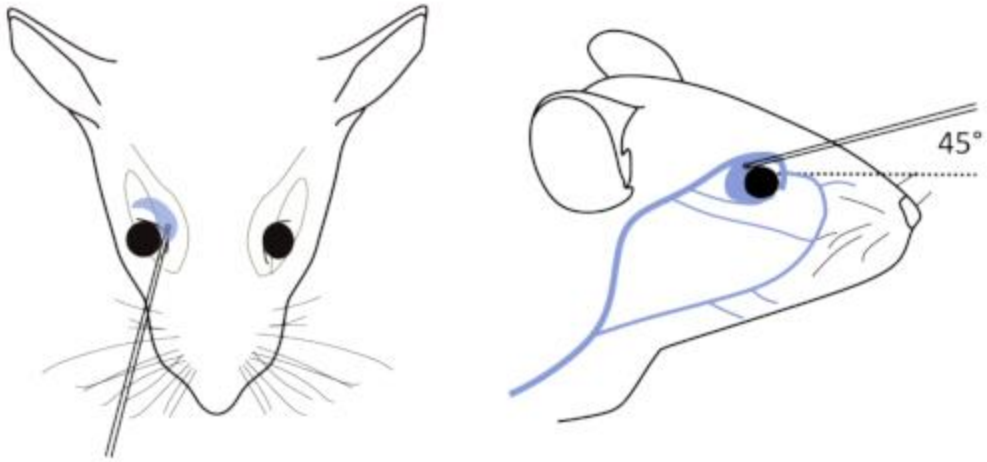
Circulatory cortisol and noradrenaline were increased within 15 min, peaked at 2 h after acute stress, and returned to baseline by 4 h; however, IL-6, which was significantly increased in blood by 2 h, peaked at 4 h, and was even detectable above baseline 18 h after acute stress (Figure 1D).



To exclude circadian oscillations leading to fluctuations in IL- 6, we measured circulating IL-6 over time by **retro-orbital bleeding and observed that repeated bleeding of the same animals sustained high IL-6 levels.** When individual cages of unmanipulated, entrained animals were bled at the corresponding Zeitgeber times (five mice per ZT time, bled only once at that ZT time), no such sustained increase in IL-6 was noted, demonstrating that conscious bleeding itself increased circulating IL-6 and that repeated bleeding sustained high IL-6 levels (Figure 1C).

Retro-orbital bleeding - OACU – NIH

Retro-orbital sampling can be used in both **mice** and rats by penetrating the **retro-orbital** sinus in **mice** or plexus in rats with a sterile hematocrit capillary tube or Pasteur pipette. Sterile tubes are recommended to help avoid periorbital infection and potential long-term damage to the eye. Good sample quality.



Still fig.1

We did not detect corresponding increases in the soluble IL-6 receptor (Figure S1A) (Khokha et al., 2013).

The absolute level of IL-6 we detected fell in the middle range of reported levels (50 pg/mL to 200 ng/mL) in inflammatory contexts and above reported ranges post-exercise and in diet-induced obesity (30–100 pg/mL

Adrenalectomized mice had significantly higher levels of IL-6 after stress, suggesting that the adrenal gland negatively regulated IL-6

We utilized an antagonistic anti-**IL-6Ra *** antibody and found that inhibition of IL-6 signaling did not change circulating levels of corticosterone or noradrenaline after acute stress (Figures S1C and S1D). This model avoids the confounding developmental defects observed in constitutive IL-6 knockout animals

***this is the same as IL-5R not necessarily in its soluble version**

Human data

We acquired a community sample of individuals that were carefully assessed for high and low stress by using a structured cumulative stress and adversity interview that assessed recent and past life events

We found significant overall increased IL-6 levels in the high (74 pg/mL, SE: 35) versus low (3.9 pg/mL, SE: 2.78) stress groups ($t = 2.15$, $p < 0.05$

The absolute circulating level of stress-associated IL-6 in humans was a hundred times lower in mice, reflecting inter-species variation and/or acuity, heterogeneity, and magnitude of stressors. Taken together, these data indicate that IL-6 is an endocrine hormone inducible by acute stress alone, with different kinetics than the canonical stress hormones, corticosterone and noradrenaline

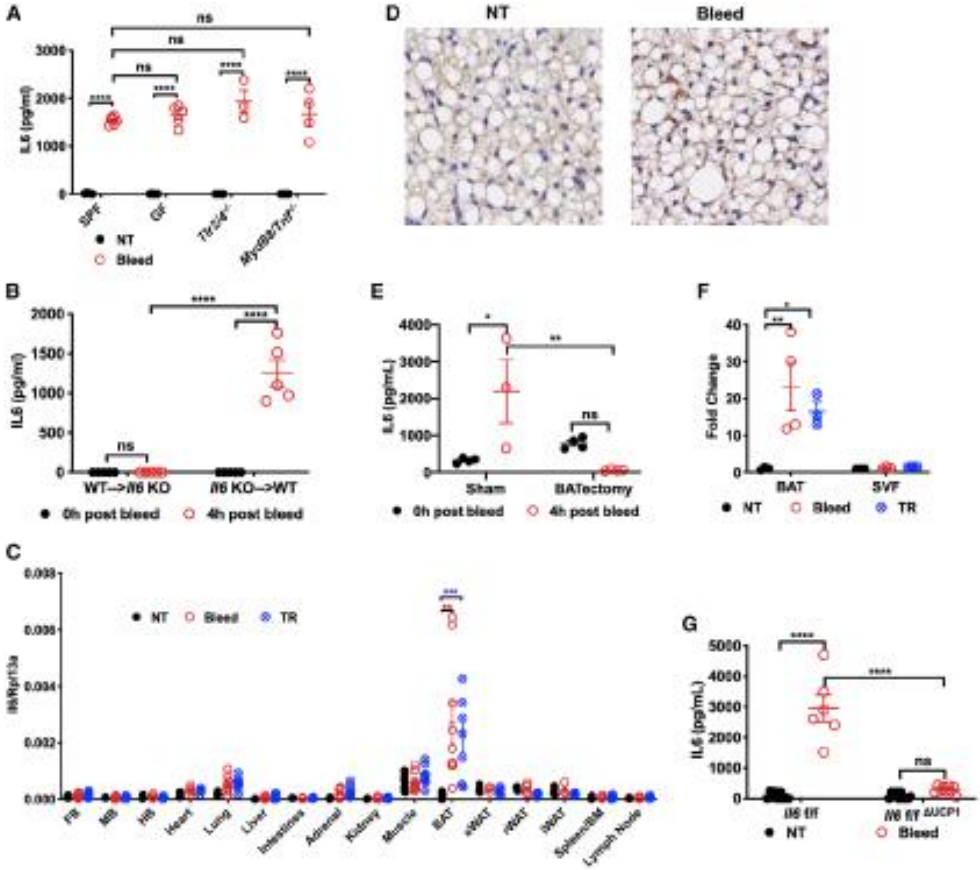
Figure 2. Stress-inducible IL-6 is produced by brown adipocytes

CONTROL EXPS

Stress-induced IL-6 was present in both gnotobiotic animals and animals deficient in key signaling pathways necessary for detecting bacteria (Figure 2A)

Acute stress of gavaging and bleeding, but not hyperglycemia, was responsible for IL-6 induction

The contribution of systemic IL-6 was not significantly affected by local damage



IL-6 is produced by many cell types, including hematopoietic cells, myocytes, endothelial cells, and adipocytes. To identify the origin of IL-6, we first performed mixed bone marrow chimera studies by using IL-6-deficient animals and found that stress-induced IL-6 was not produced by radiosensitive cells (KILLED BY RADIATION, Figure 2B). we screened multiple tissues for Il6 induction using both the bleeding and tube restraint models. We found that Il6 was robustly induced in the brown adipose tissue (BAT) (Figure 2C and D)

Still fig.2

BAT (BROWN ADIPOSE TISSUE) produces IL-6 AFTER STRESS

To test if BAT was the sole source of stress-induced IL-6, we surgically excised the BAT, which ablated the IL-6 response to bleeding stress (Figure 2E).

Because BAT is a complex collection of cells including radioresistant immune cells, we first asked if stress-induced Il6 would be present in the **stromal vascular fraction (SVF),** which includes **all cells except adipocytes**. Il6 transcriptional induction was not observed in the SVF fraction from either bled or restrained animals, implying that stress-induced IL-6 was derived from brown adipocytes (Figure 2F)

we generated an animal in which Il6 could be inducibly deleted in brown adipocytes by using Ucp1 **(THIS IS A BAT MARKER)** promoter-driven Cre under the control of estrogen receptor (Il6f/fDUCP1) and detected a significant attenuation of IL-6 after acute stress in these animals (Figure 2G). Collectively, our data demonstrate that brown adipocytes are the source of stress-induced IL-6.

Figure 3. ADRB3 mediates brown adipocyte-derived IL-6 in response to acute stress

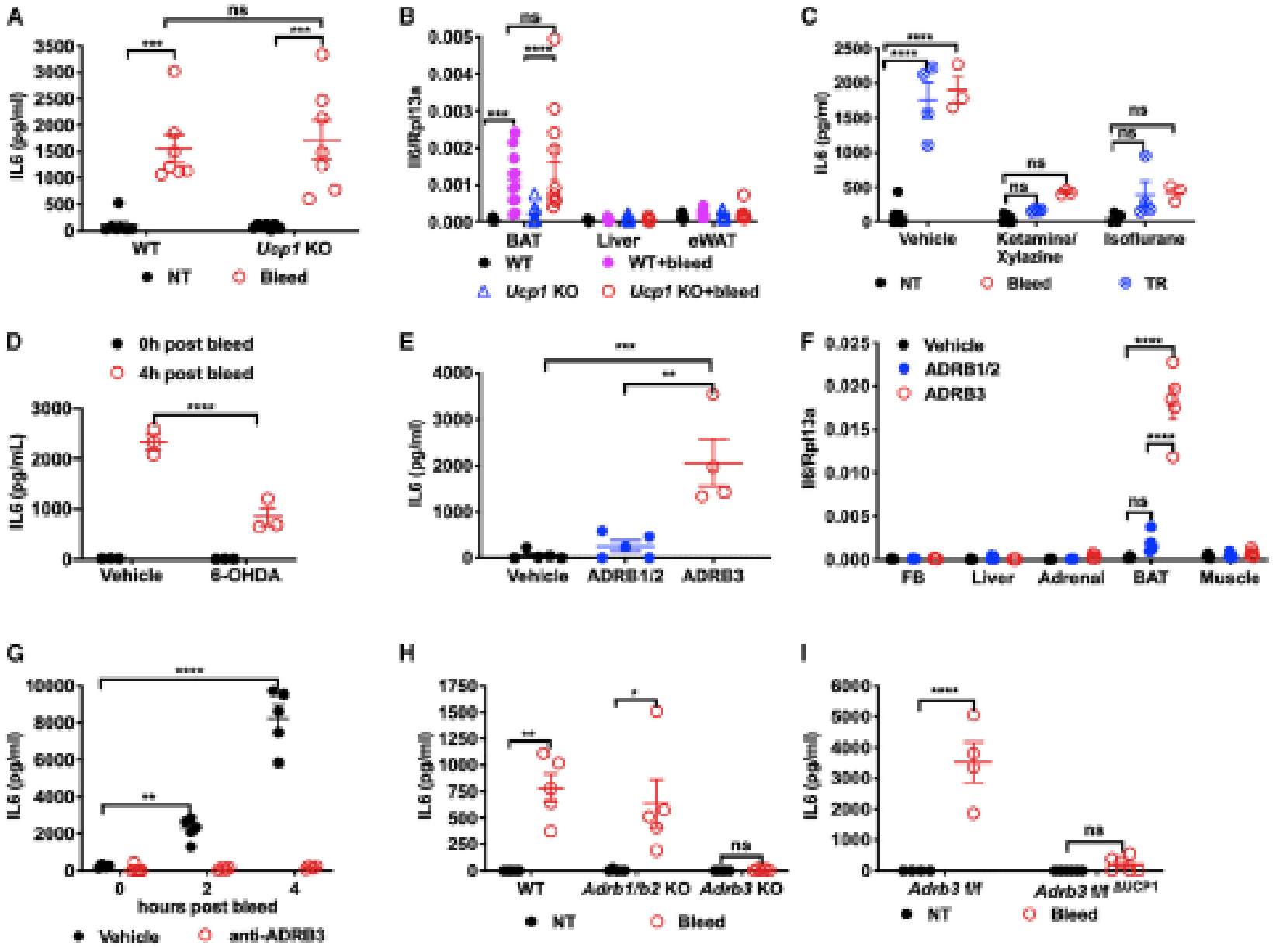


Fig.3

Animals need to be conscious to produce IL-6

3A and B are controls

We reasoned that consciousness would be required for IL-6 response to acute stress. Thus, we anesthetized animals with either ketamine/xylazine or isoflurane, after which we subjected them to tube restraint or retro-orbital bleeding and found that anesthesia abrogated stress-induced IL-6 (Figure 3C).

NA is necessary for stress induced BAT production of IL-6

We utilized 6-hydroxydopamine (6-OHDA) at doses that achieve significant BAT sympathectomy without significant effects on the CNS (Depocas et al., 1984). After 6-OHDA treatment, we found that stress-inducible IL-6 was significantly attenuated, confirming that sympathetic outflow was required (Figure 3D).

Fig 3E-I show that it is NAR beta 3 (and not 1 or two) the responsible for BAT IL-6 release

IL-6 is necessary for maintaining hyperglycemia after acute stress

Fig.4

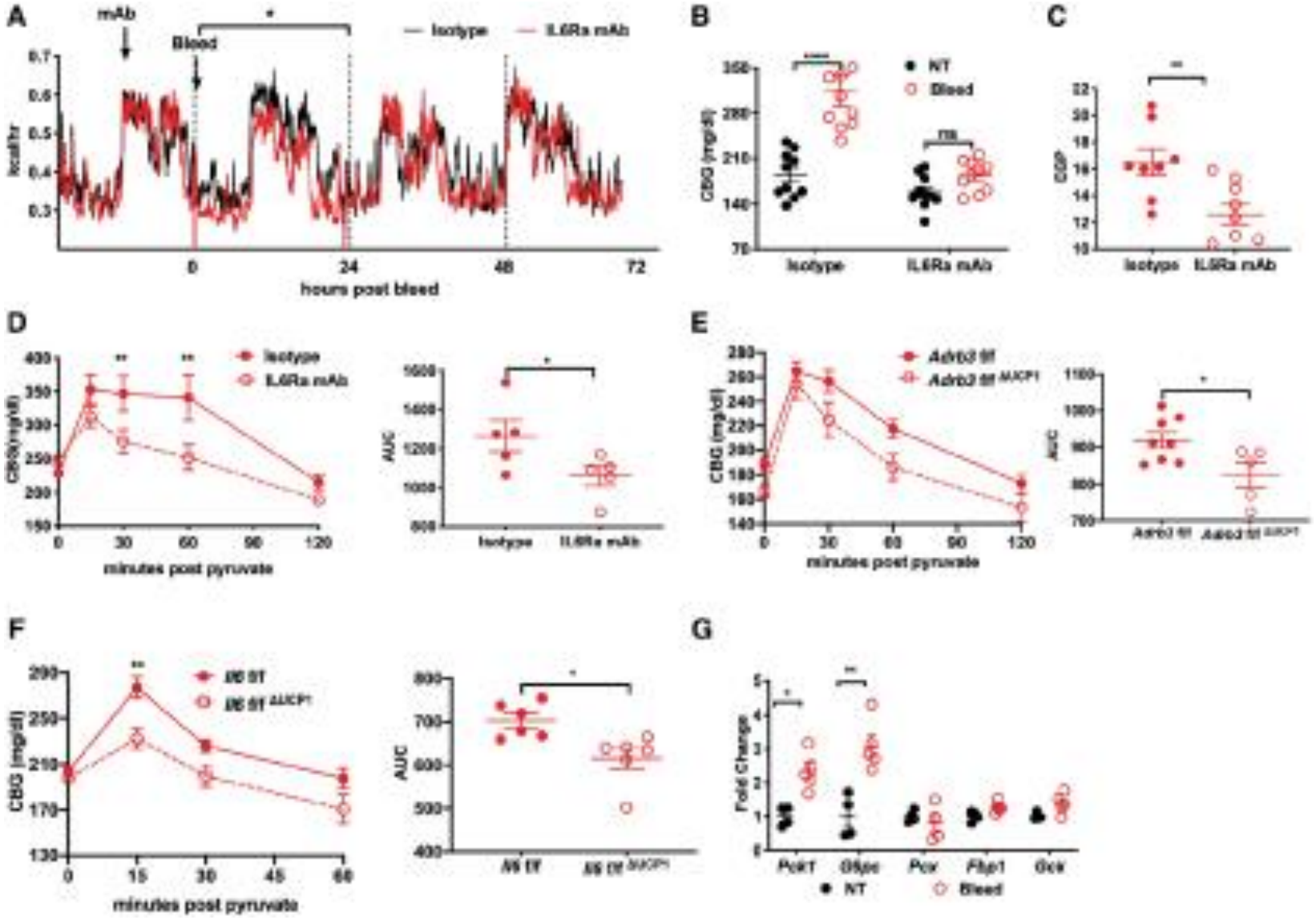


Fig. 4

Acute stress alleviated insulin-induced hypoglycemia at later time points (Figure S2G), suggesting that stress-induced IL-6 might potentiate endogenous glucose production

Significant **differences exist in the overall energy expenditure of stressed animals in which IL-6 signaling was antagonized vs. control** (Figure 4A) despite no significant changes in total activity.

Stress-induced hyperglycemia was durable at 4 h after acute stress in an IL-6Ra-dependent fashion (Figure 4B).

Hyperglycemia is caused by impaired clearance (insulin resistance) and/or excess glucose production from **glycogenolysis or gluconeogenesis**. There is no effect of IL-6 on insulin-dependent glucose uptake.

Acute stress alleviated insulin-induced hypoglycemia at later time points (Figure S2G), suggesting that

stress-induced IL-6 might potentiate endogenous glucose production

A **single dose of recombinant IL-6 was also sufficient to recapitulate the effects of acute stress** on maintaining higher levels of glucose at late time points during ITT (Figure S2H), and IL-6Ra blockade in stressed animals attenuated the ability of mice to maintain normoglycemia after insulin challenge .

IL-6 was inducing gluconeogenesis during stress

Still fig 4

Endogenous glucose production after acute stress was significantly decreased in the absence of IL-6 signaling (Figure 4C).

This finding was supported by pyruvate tolerance tests (PTT) in stressed animals, where **pyruvate conversion to glucose was impaired in the absence of IL-6Ra signaling** (Figure 4D).

Consistent with our previous findings, we found that **the effects of IL-6Ra antagonism could be fully recapitulated by using Il6f/fDUCP1 and Adrb3f/fDUCP1 animals, which lack stress-inducible IL-6** (Figures 4E and 4F).

Gluconeogenic capacity is mediated by key rate-limiting enzymes, many of which have been shown to be sensitive to IL-6 signaling via STAT3 regulatory elements.

We thus assessed the hepatic transcriptional induction of Pck1, G6pc, and other **gluconeogenic genes and found that Pck1 and G6pc were significantly increased after acute stress** (Figure 4G)

Figure 5. IL-6 mediates stress hyperglycemia through hepatocyte reprogramming

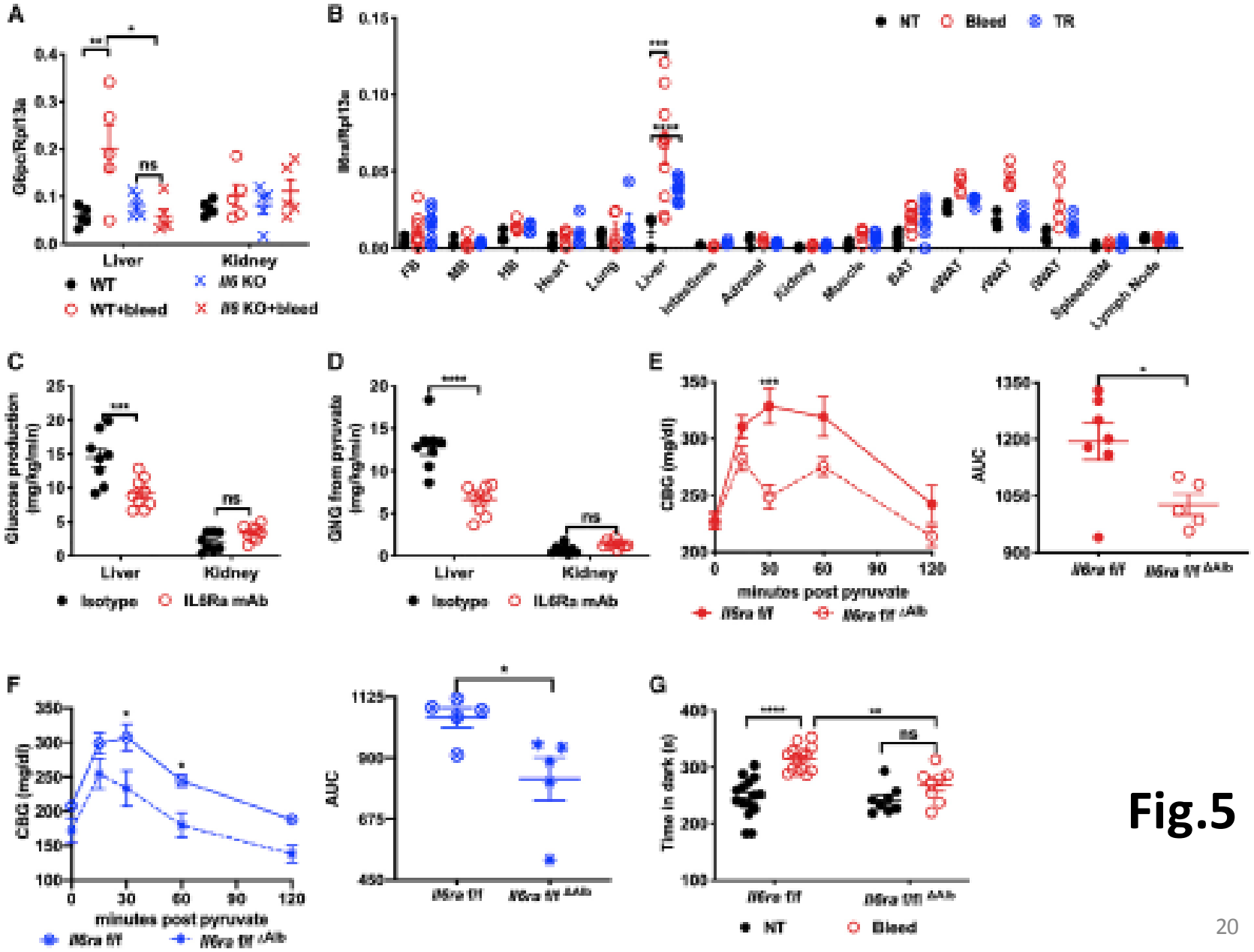


Fig.5 stress-inducible IL-6 acts on the liver to induce hepatic gluconeogenesis.

transcriptional induction of gluconeogenic genes in kidney and liver were induced by bleeding stress only in the **liver** (Figures 5Ain liver but not in kidney (Figure 5B)

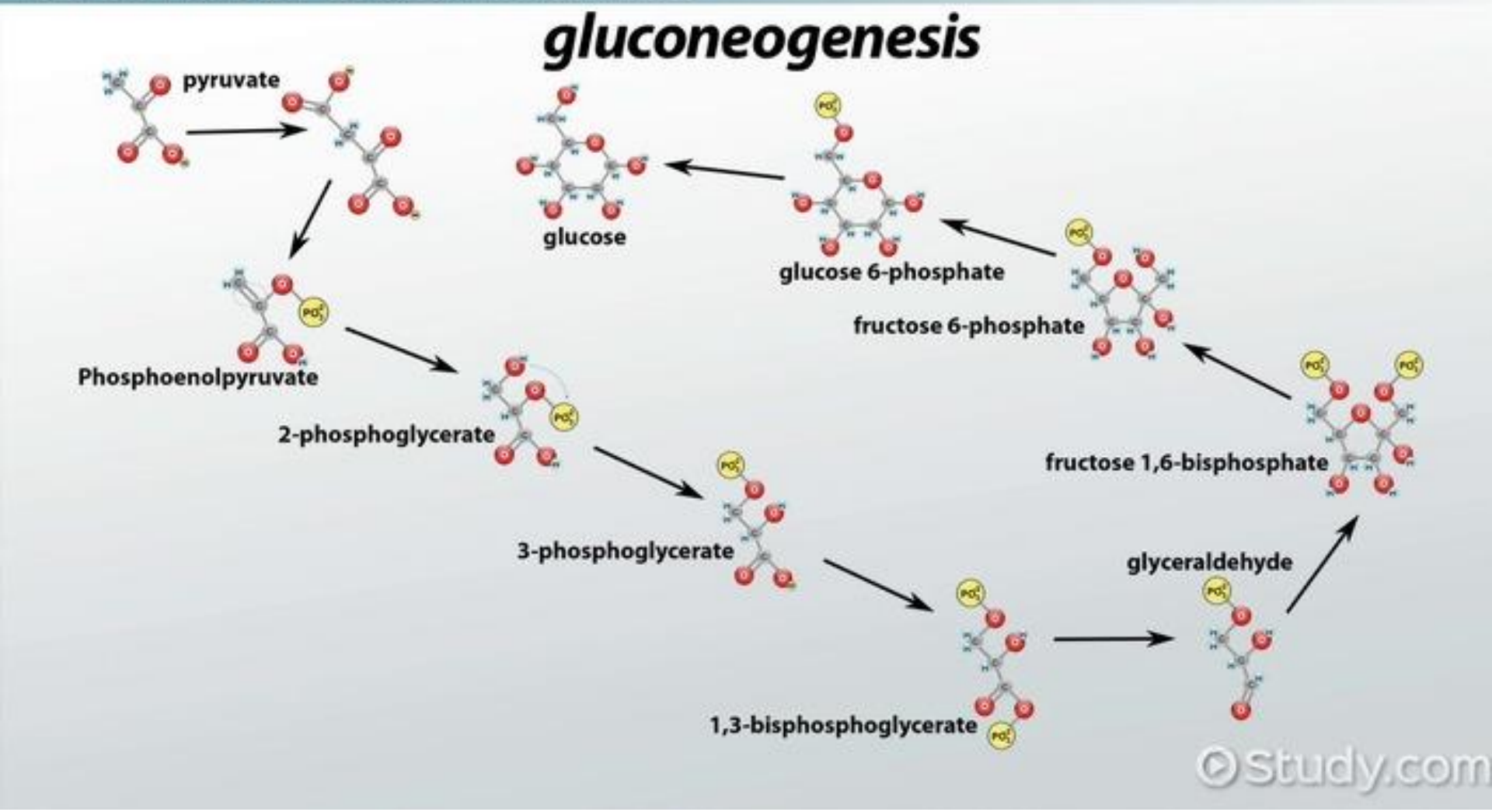
IL-6Ra in the liver controls stress hyperglycemia through hepatocyte reprogramming suggesting that the **liver might be the primary target for IL-6 signaling in response to acute stress.**

IL-6Ra antibody suppressed gluconeogenesis from pyruvate in liver but not in kidney (Figures 5C and 5D).

Finally, to directly assess the role of hepatocyte IL-6Ra, we generated mice with hepatocyte-specific deletion of Il6ra and performed Pyruvate tolerance test PTT after acute stress. We found that hepatocyte-specific deletion was sufficient to recapitulate the inhibitory effects of systemic IL-6Ra blockade on gluconeogenesis after bleeding (Figure 5E) and restraint stress (Figure 5F).

Pyruvate Tolerance Test (PTT) *is used to assess gluconeogenesis. During a fasting state, hypoglycemia is normally prevented via two hepatic processes: glycogenolysis (the degradation of glycogen) and gluconeogenesis (the generation of glucose from non-carbohydrate carbon substrates including pyruvate and lactate). The Pyruvate Tolerance Test is a variant of the GTT in which pyruvate is injected instead of glucose. The pyruvate bolus will elicit a glycemic excursion that will reflect the hepatic gluconeogenesis.*

DEFINITION OF GLUCONEOGENESIS



Still fig 5

We reasoned that **the purpose of activating gluconeogenesis during acute stress, when animals are neither hypoglycemic nor in net-negative energy balance states, is anticipatory of impending increased demand ("fight or flight" response**).

Consequently, **impairment of hepatic gluconeogenesis should be sufficient to affect adaptive behavioral responses to acute stress**.

To test this, we utilized **the light-dark box paradigm**.

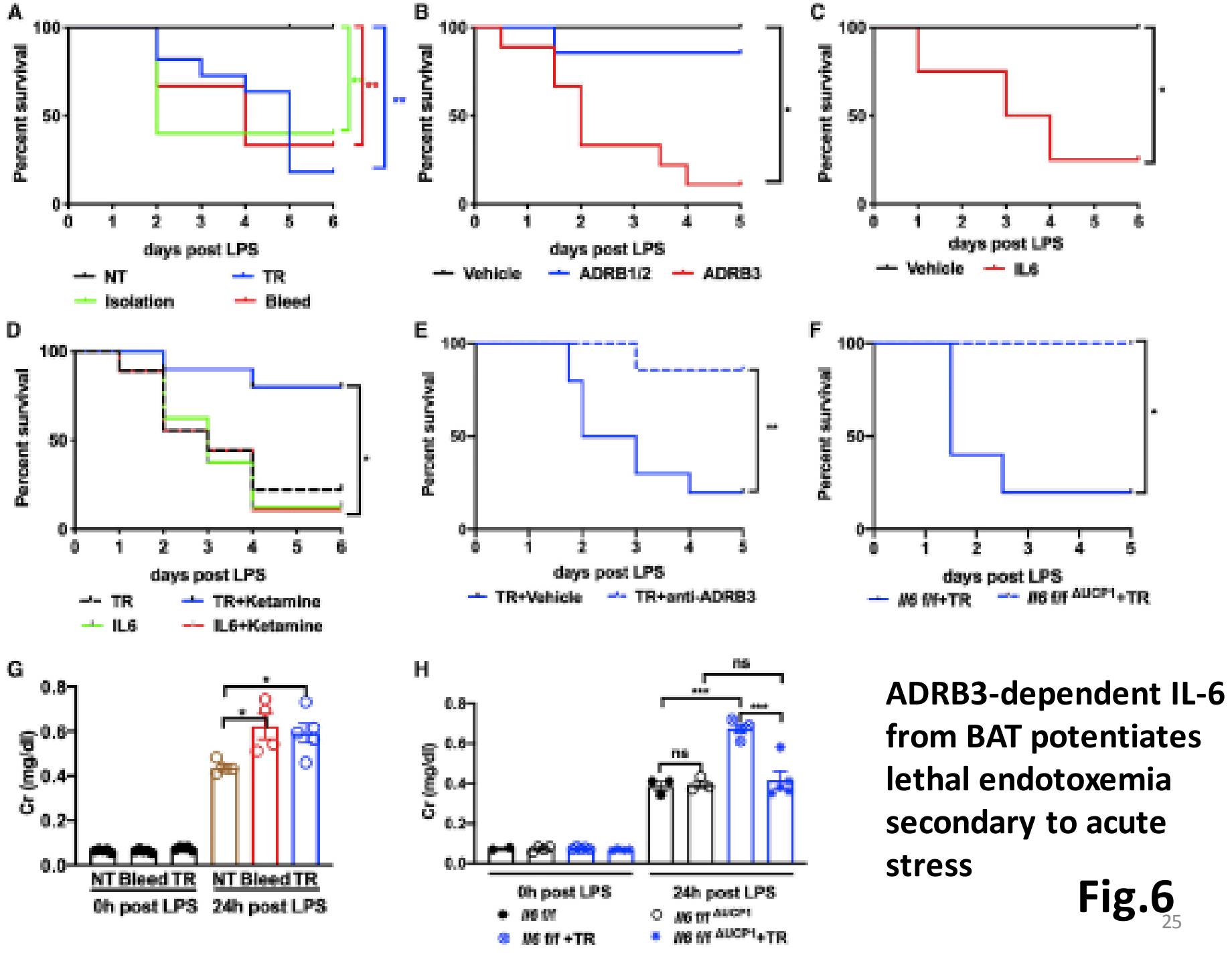
light-dark box

The light-dark box paradigm is a common tool for studying stress response behaviors where animals are placed into a **novel environment in which part of the apparatus is exposed under bright light and connected to another enclosed and opaque space by a small opening.**

In this paradigm, **animals must balance the need to explore the novel space with the fear of avoiding possible predation in the exposed area**.

A normal adaptive response is to spend more time in the dark enclosure. We thus established baseline responses of **animals with conditional hepatic deletion of Il6ra** and then **compared the responses at the peak of endocrine IL-6 after a single, conscious, retro-orbital bleed**.

We found that **hepatic IL-6Ra was required for the normal behavioral response** (Figures 5G and S4F). Taken in aggregate, we demonstrate that **stress-induced IL-6 mediates stress-hyperglycemia through hepatic IL-6Ra signaling in positive energy balance states, and hepatic IL-6Ra is necessary for a normal behavioral response to acute stress**



ADRB3-dependent IL-6 from BAT potentiates lethal endotoxemia secondary to acute stress

Fig.6

Priming animals with stress robustly enhanced mortality to LPS (Figure 6A).

For the LPS studies, we opted to use the tube-restraint model to avoid confounders associated with the hemodynamic consequences of bleeding.

Fig 6

pre-treatment with ADRB3 agonist alone was sufficient to enhance LPS mortality (Figure 6B). Concordantly, **a single injection of stress-dosed IL-6 was sufficient to potentiate LPS-induced mortality** (Figure 6C).

Because stress-induced IL-6 required consciousness, we tested whether or not animals anesthetized prior to tube restraint were still more susceptible to LPS-induced mortality and found that **consciousness was required for the stress-priming effect, an effect that could be bypassed with endogenous administration of IL-6** (Figure 6D).

To test if ADRB3-dependent IL-6 was necessary, **ADRB3 antagonist** was applied alongside the restraint challenge, which negated the effects of stress-priming (Figure 6E).

Finally, we asked if Il6f/fDUCP1 animals, which **lack stress-inducible IL-6**, would be resistant to stress-priming (Figure 6F). Consistant with this hypothesis , Il6f/fDUCP1, which did not display altered susceptibility to LPS in the absence of stress-priming (Figure S5A), was resistant to the potentiating effects of tube-restraint on LPS mortality

We also tested the effects of stress-priming by using our Il6raf/fDAlb model. Here, regardless of stress-priming, **animals lacking hepatic IL-6 signaling were significantly more sensitive to endotoxemia, suggesting that the hepatic acute phase response was a required adaptation to endotoxemia**

Still fig 6

Because end-organ dysfunction is a hallmark of inflammatory damage, we assessed biomarkers of vital organ function in stress-primed versus control animals and found that **stress-primed animals displayed significantly more renal and a trend toward more cardiac damage, whereas hepatic damage appeared to be equivalent across conditions (Figures 6G, S6G, and S6H)**.

These markers of end-organ damage were absent in stress-primed Il6f/fDUCP1 animals (Figure 6H), demonstrating that **BAT-derived IL-6 from stress was required for decreasing tolerance to inflammatory damage.**

Together, these findings suggest that

stress decreases host tolerance to inflammation in a BAT-derived IL-6-dependent fashion.

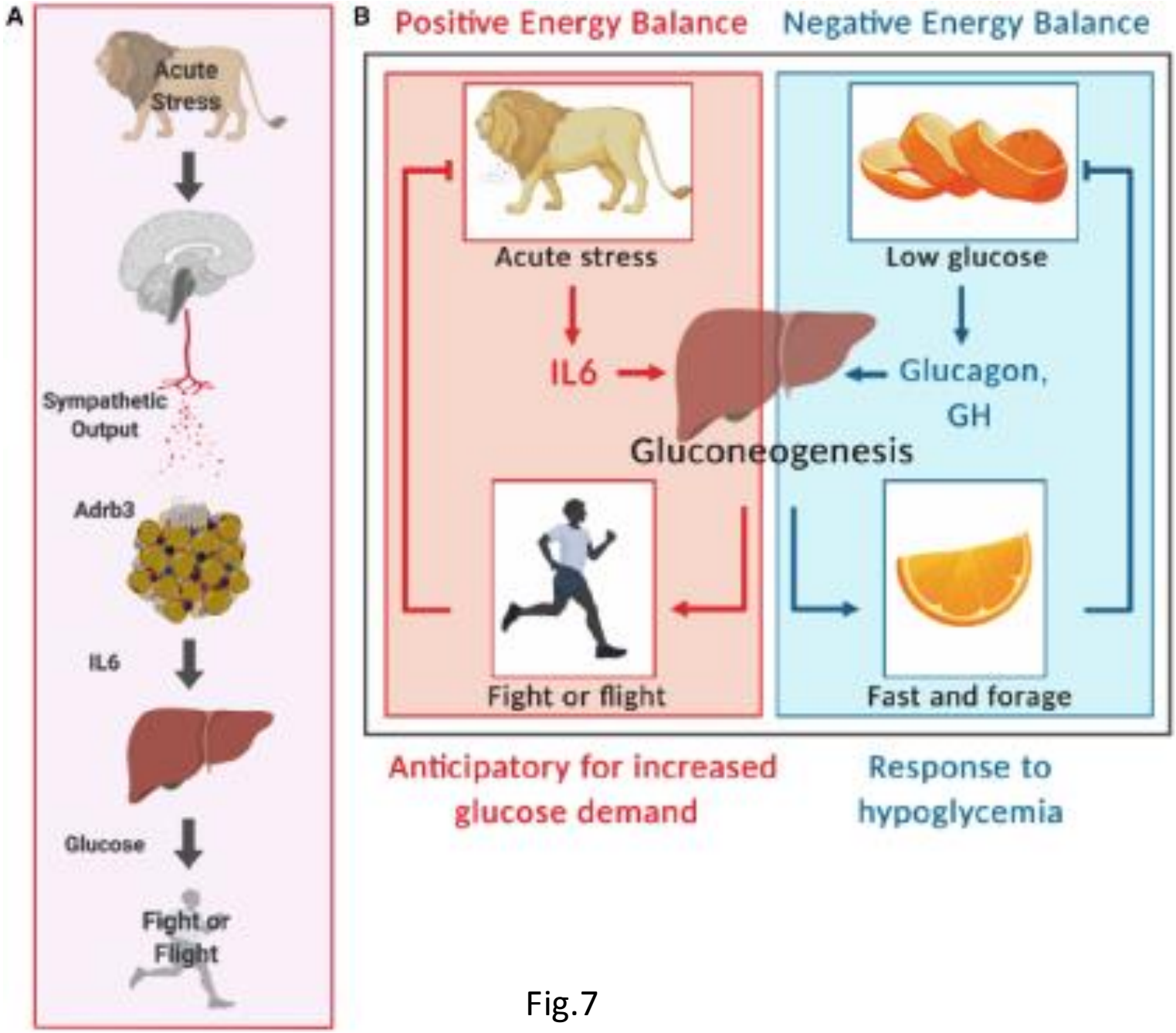

A
Acute Stress
Sympathetic Output
Adrb3
IL6
Glucose
Fight or Flight
B
Positive Energy Balance
Negative Energy Balance
Acute stress
IL6
Fight or flight
Low glucose
Glucagon, GH
Gluconeogenesis
Fast and forage
Anticipatory for increased glucose demand
Response to hypoglycemia

Fig.7

CONCLUSIONS

Stress-induced IL-6 is produced from brown adipocytes in an ADRB3-dependent fashion in mice. Thermogenic programs were not engaged in this context, and this response was independent of ambient temperature.

One key role of stress-induced endocrine IL-6 is in reprogramming organismal metabolism by instructing hepatic gluconeogenesis in the absence of a net negative energy balance or hypoglycemic state, likely in anticipation of increased glucose demand.

Hepatic IL-6 signaling was also necessary for mediating normal behavioral responses in the light-dark box paradigm suggesting that hepatic organismal reprogramming is required for an adaptive ‘‘fight or flight’’ response.

Finally, stress-induced BAT-derived endocrine IL-6 was necessary and sufficient for decreasing host tolerance to a subsequent inflammatory response by using the endotoxemia model.

The precise mechanism by which stress decreases host fitness to inflammation remains to be understood. The study suggest that stress-induced IL-6, although adaptive for supporting fight-or-flight physiology, comes at the cost of decreasing host fitness to endotoxemia-induced inflammation.