High-fat Feeding Promotes Obesity via Insulin Receptor/PI3k-Dependent Inhibition of SF-1 VMH Neurons

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Supplementary Figure Legends

Supplemetary Figure 1: Unaltered number of SF-1-expressing neurons in SF-1$^{\Delta IR}$-mice.

Total number of LacZ-positive SF-1 neurons in the VMH of SF-1^{LacZ} (control) and SF-1$^{LacZ:\Delta IR}$-mice (n=8-10 mice per genotype).

Data represent the mean $\pm$ S.E.M.

Supplementary Figure 2: Unaltered fertility and pituitary function in SF-1$^{\Delta IR}$-mice.

a) Average litter size of female control and SF-1$^{\Delta IR}$-mice (n=10-14 breedings per genotype).

b) Litter frequency of female control and SF-1$^{\Delta IR}$-mice (n=10-14 breedings per genotype).

c) Pituitary gland mRNA-expression of follicle stimulating hormone (FSH), luteinizing hormone (LH), thyroid stimulating hormone (TSH) and growth hormone (GH) (n=8 per genotype).

d) Serum levels of free T_3 in NCD and HFD-exposed control and SF-1$^{\Delta IR}$-mice at the age of 20 weeks (n=15 per genotype).

Data represent the mean $\pm$ S.E.M.

Supplementary Figure 3: Food intake adjusted for body weight is unaltered in older SF-$1^{\Delta IR}$-mice.

Food intake of control and SF-$1^{\Delta IR}$-mice at the age of 12 to 13 weeks on HFD normalized to body weight (n>14 per genotype).

Data represent the mean ± S.E.M.

Supplementary Figure 4: Unaltered glucose metabolism of control and SF-$1^{\Delta IR}$-mice on NCD.

a) Blood glucose concentrations of random fed control and SF-$1^{\Delta IR}$-males on NCD at the age of 20 weeks (n>14 per genotype).

b) Serum insulin concentrations of male control and SF-$1^{\Delta IR}$-mice exposed to NCD at the age of 20 weeks (n>14 per genotype).

c) Insulin tolerance test of 14 weeks old male control and SF-$1^{\Delta IR}$-mice on NCD (n>14 per genotype).

d) Glucose tolerance test of 15 weeks old male control and SF-$1^{\Delta IR}$-mice on NCD (n>14 per genotype).

Data represent the mean ± S.E.M.

Supplementary Table Legends

Supplementary Table 1: Electrophysiological responses of SF-1 VMH neurons.

Recordings were performed in genetically marked SF-1 neurons. Summarized are responses to application of either leptin or insulin. Note that recordings were performed throughout the VMH, while insulin responses depicted in Fig. 2 were performed in the mediobasal VMH, where insulin evoked clear PI3k activation, explaining the higher percentage of insulin-responsive cells there. Recordings were performed in 70 neurons before and after leptin stimulation and in another 38 neurons following insulin stimulation. Observed depolarization by leptin reached on average +5 mV and was usually reversible, while the hyperpolarization by leptin or insulin reached on average -7 mV and was usually irreversible.

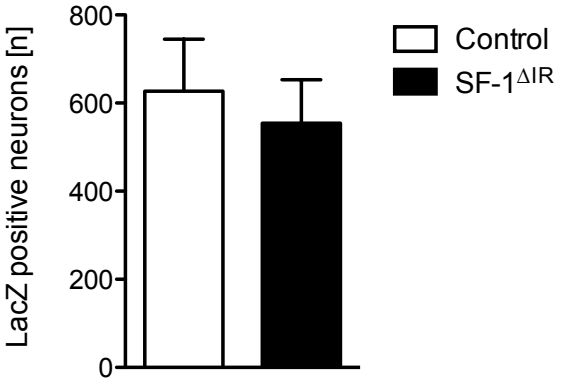
Supplementary Table 2: Effect of sequential leptin and insulin application on firing properties of SF-1 VMH neurons.

Recordings were performed in genetically marked SF-1 neurons. Summarized are responses to sequential application leptin followed by insulin. Note that 3 out 21 neurons not responding to leptin hyperpolarized upon subsequent insulin treatment, while 2 neurons, which depolarized upon leptin treatment fail to respond to subsequent insulin incubation, indicating that 5/21 neurons tested in this paradigm exhibit segregated responses to leptin and insulin.

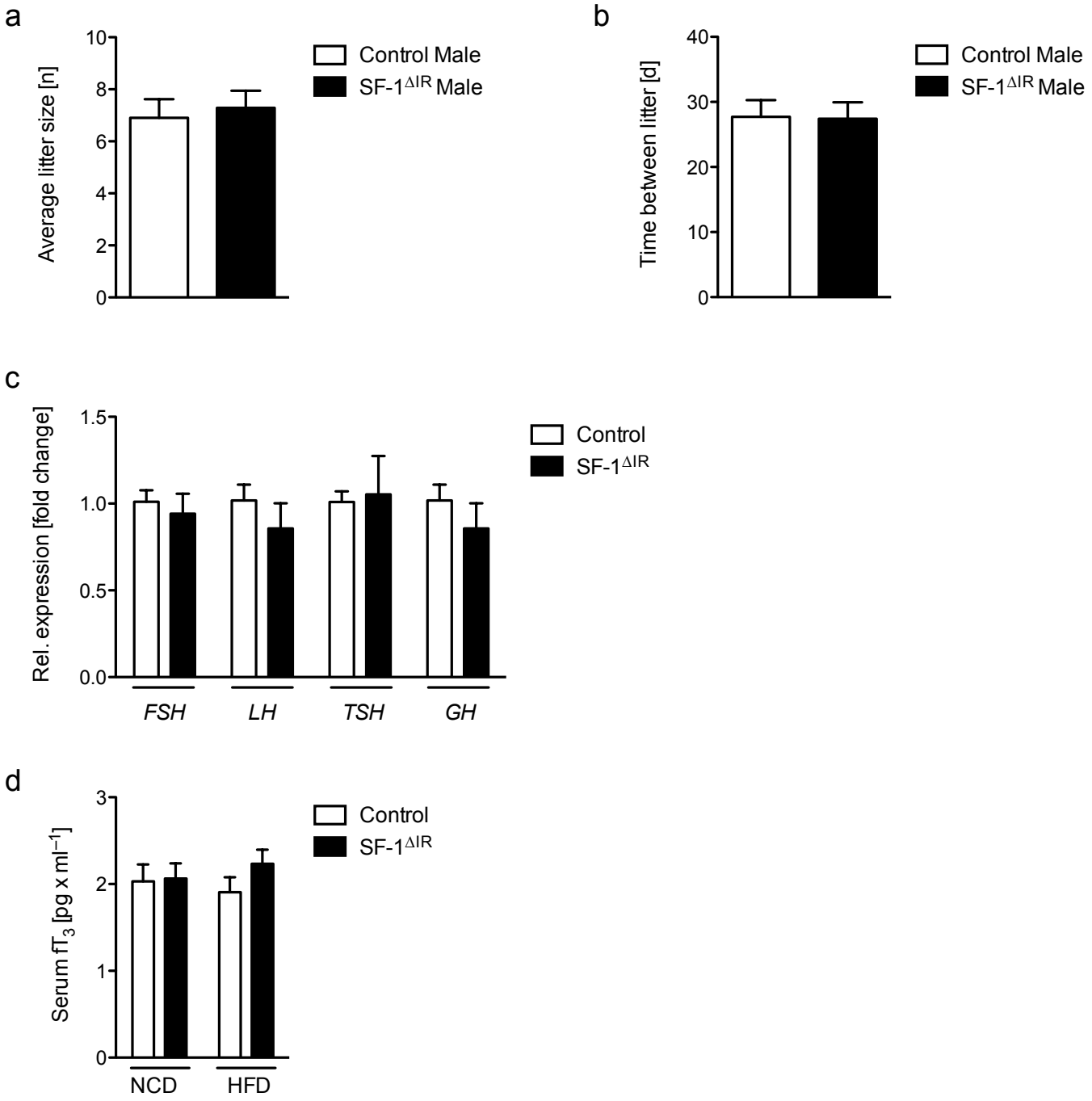
Supplementary Table 3: Effect of sequential insulin and leptin application on firing properties of SF-1 VMH neurons.

Recordings were performed in genetically marked SF-1 neurons. Summarized are responses to sequential application insulin followed by leptin. Note that out of 16 neurons not responding to insulin upon first incubation, 1 depolarized and 5 hyperpolarized upon subsequent leptin treatment, indicating that 6/16 neurons tested in this paradigm exhibit segregated responses to leptin and insulin.

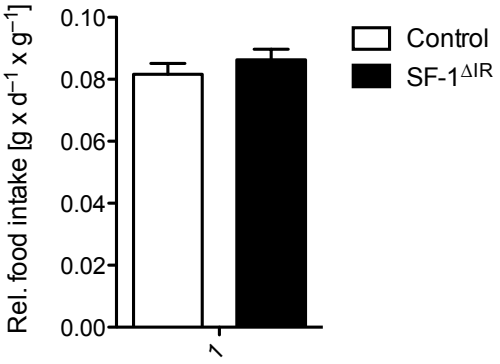
Klöckener et al. Supplementary Figure 1



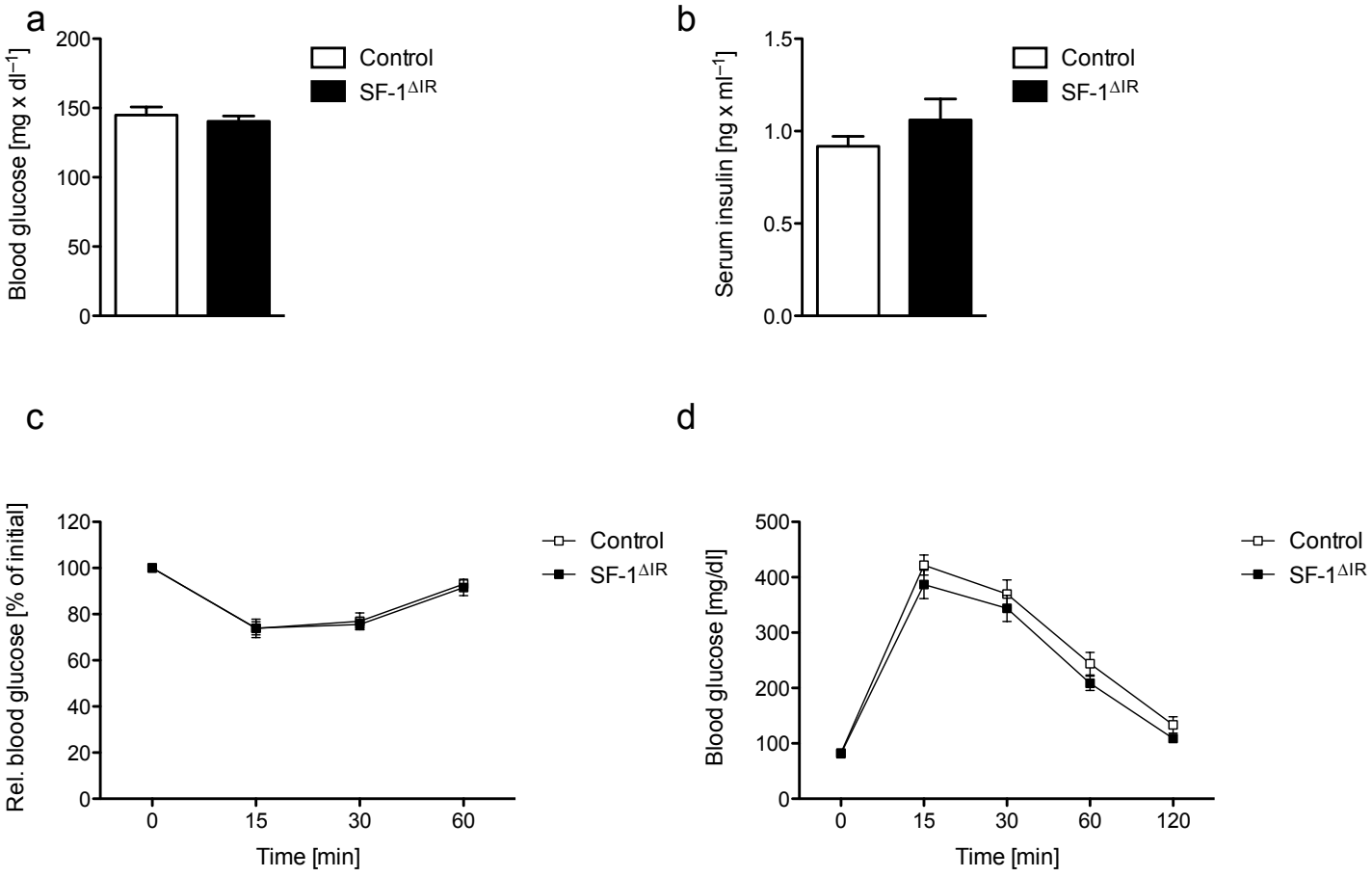
Klöckener et al. Supplementary Figure 2



Klöckener et al. Supplementary Figure 3



Klöckener et al. Supplementary Figure 4



Klöckener et al. Supplementary Tables

Table 1

	Leptin	**Insulin**
Depolarized	10 (13.3 %)	0 (0 %)
Hyperpolarized	15 (20.0 %)	5 (12.2 %)
No response	50 (66.7 %)	36 (87.8 %)
Total	75 (100 %)	41 (100 %)

Table 2

		Leptin 1st	
		Depolarized	No response
Insulin 2nd	Hyperpolarized	0	**3**
	No response	**2**	18
	Total	2	21

Table 3

		Insulin 1st
		No response
Leptin 2nd	Depolarized	**1**
	Hyperpolarized	**5**
	No response	10
	Total	16