ELLEGAARD
GÖTTINGEN MINIPIGS

Welcome to the webinar:

Induction of prediabetes and diabetes in adult obese Göttingen Minipigs

15 April 2021

Guest speaker: Sietse-Jan Koopmans | Wageningen University & Research, The Netherlands

- Many have signed up for this webinar from around the world and therefore all attendees are muted to avoid background noises, delays in sound, echoes etc.
- Please ask your questions in the questions/chat section and we will follow up in the Q&A session following the presentation.
- We encourage everyone to complete the survey after the webinar, so we can continue planning relevant, educational and insightful webinars.
- Presentation slides and a recording of the webinar will be shared within 1-2 days via email.
- Certificates of attendance are available upon request. Please email events@minipigs.dk

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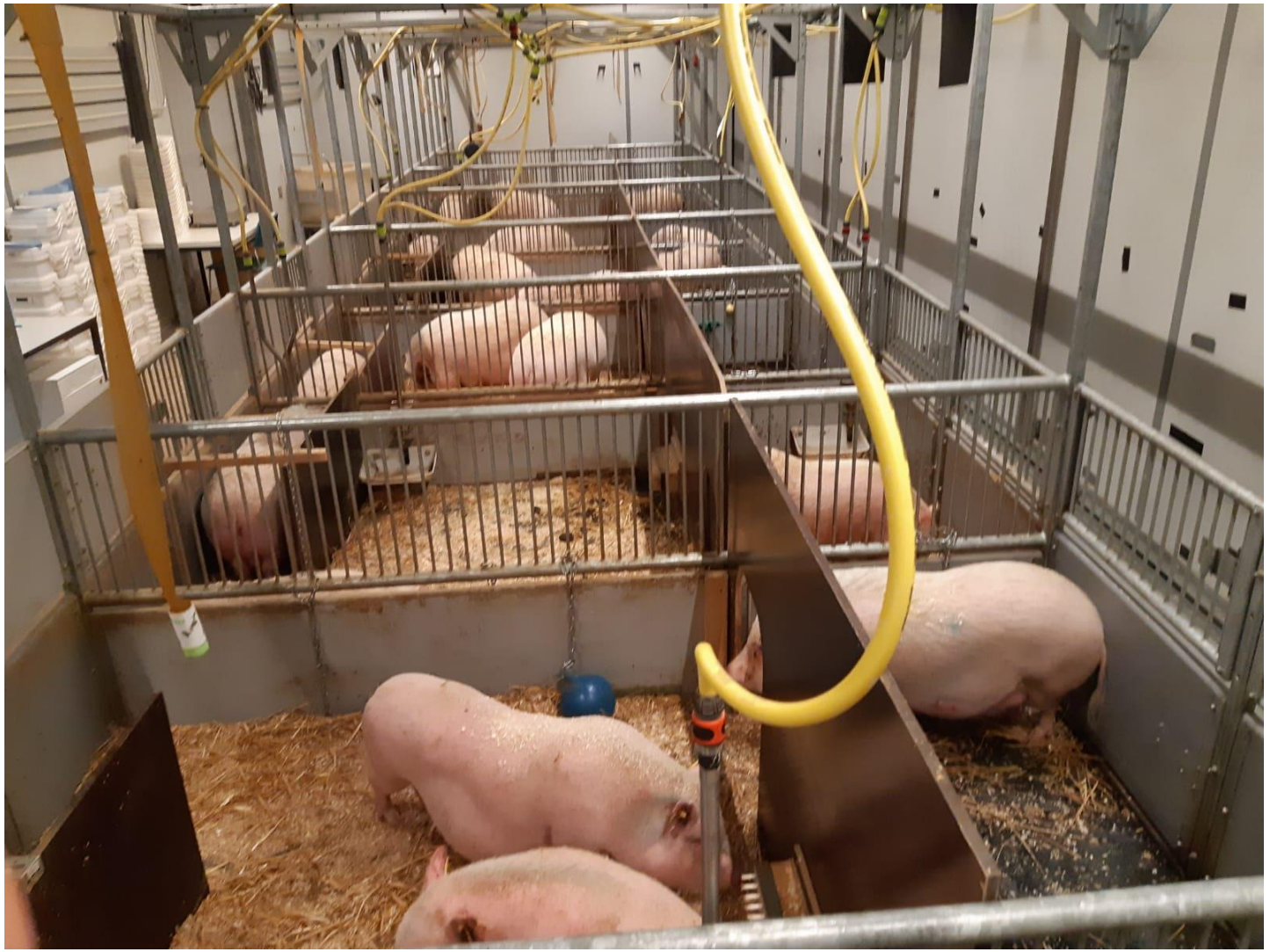
Abstract of webinar

- To follow the natural course of obese type 2 diabetes in an adult Göttingen Minipig model, the pig needs to become obese first and subsequently develop diabetes. Once insulin-resistant obesity is established in adult Göttingen Minipigs by ad libitum feeding of a high energy Western diet, diabetes should evolve after a certain period of time. The capacity of pancreatic beta-cells to produce sufficient insulin to compensate for insulin resistance becomes limited and, as a result, blood glucose concentrations increase. However, pigs do not easily develop (pre)diabetes spontaneously, at least not in the time frame of 1 or 2 years after the induction of severe obesity. To accelerate the development of diabetes in obese Göttingen Minipigs, streptozotocin can be used to chemically reduce the pancreatic beta-cell mass in order to elevate blood glucose concentrations. In the present webinar, various strategies to induce prediabetes and diabetes in adult obese Göttingen Minipigs by repeated intravenous administration of streptozotocin will be discussed.

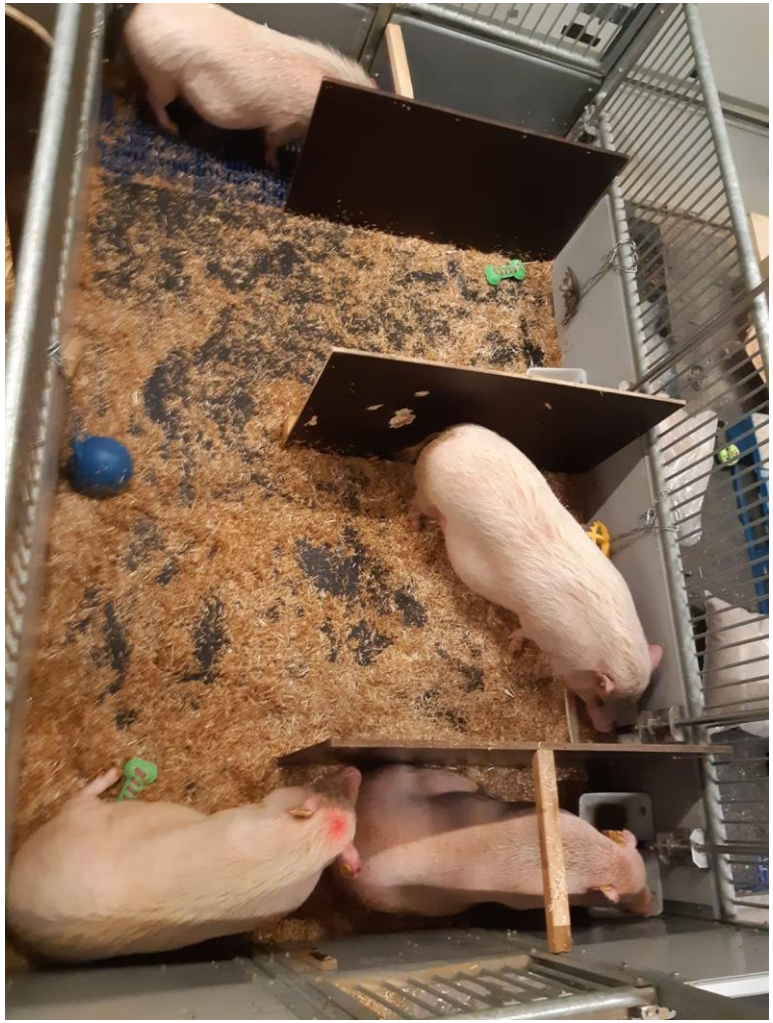
Obese Göttingen Minipigs (~70 kg)

Group housing of Göttingen Minipigs



Group (n=3) housing of Göttingen Minipigs

Group (n=4) housing of Göttingen Minipigs



Meal feeding in Göttingen Minipig

Meal feeding in Göttingen Minipig

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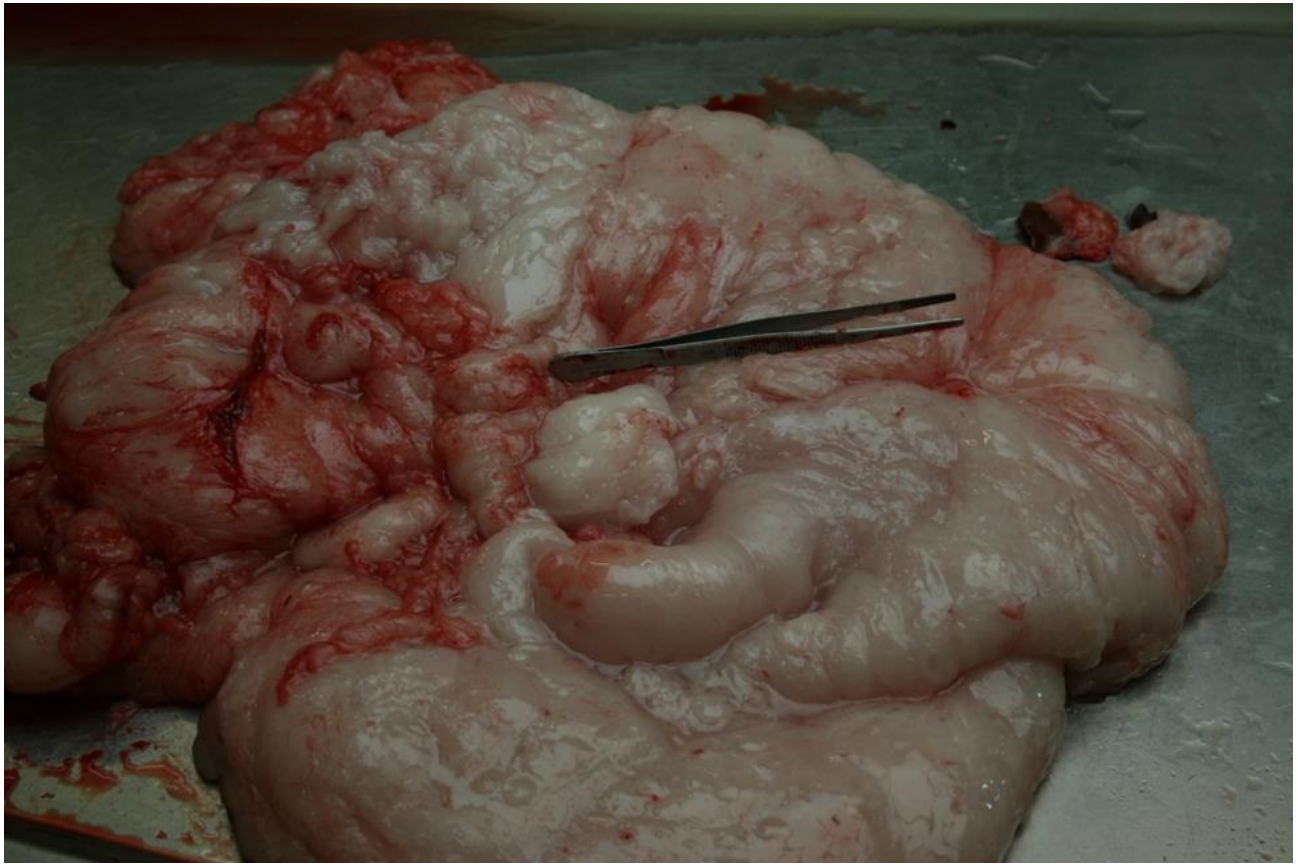
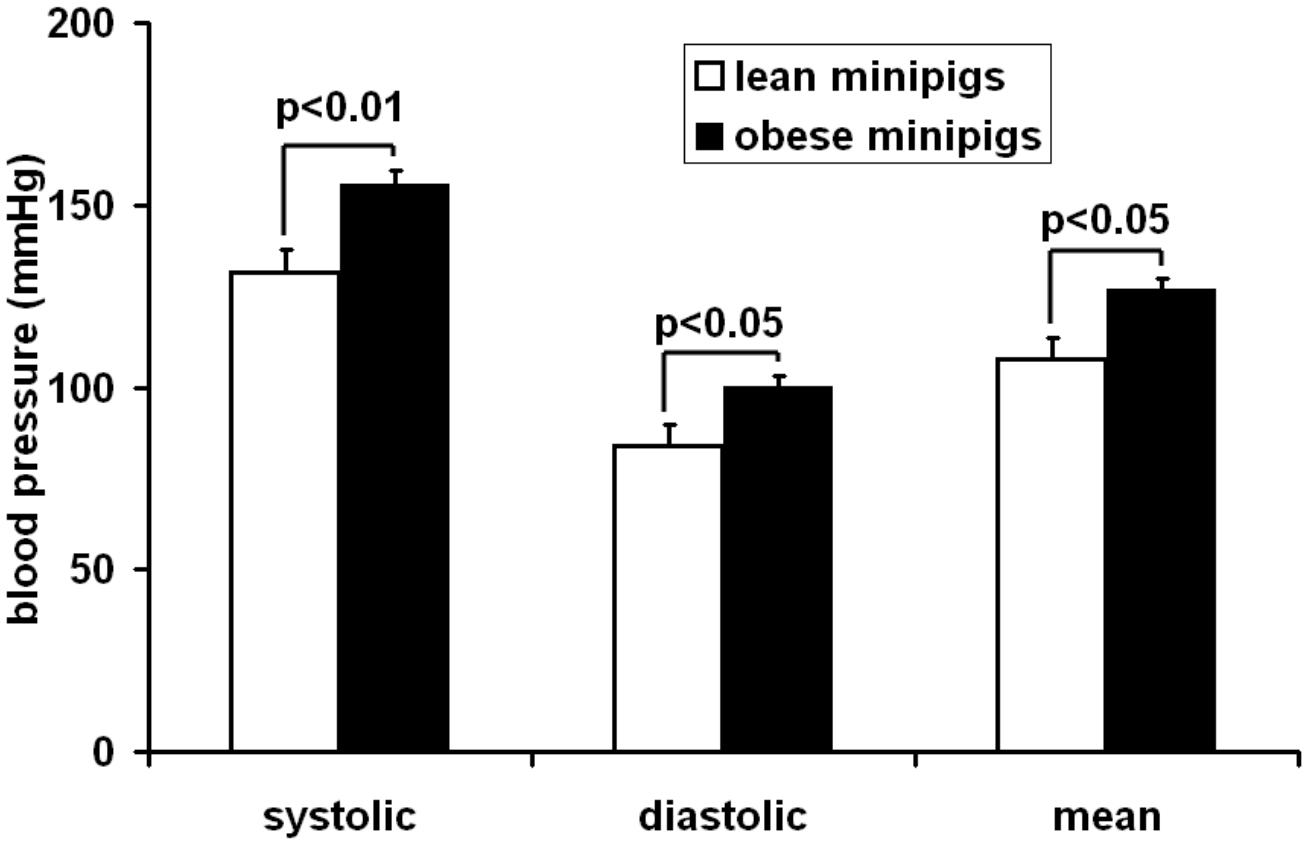
Strategy 1: no streptozotocin. Metabolic syndrome phenotype

- Many previous publications. Easy model.
- Adult (2-4 years of age) female Göttingen Minipigs (30-40 kg) are fed an obesogenic diet (i.e. 12-25% lard, 20-40% sucrose, 0.5-2% cholesterol) for periods up to 4-6 months. Body weight increases >2-fold.
- No side effects (complications) are observed
- General phenotype: elevated plasma glucose (+10-20%), insulin (up to 2-fold), cholesterol. Insulin resistance (up to 2-fold), early atherosclerosis, elevated blood pressure (+10%). Visceral fat (several-fold increase) and fatty organs.

Visceral fat, contributor to unhealthy metabolic phenotype in adult obese Göttingen minipigs on an obesogenic diet?



Blood pressure in adult obese Göttingen minipigs on an obesogenic diet.



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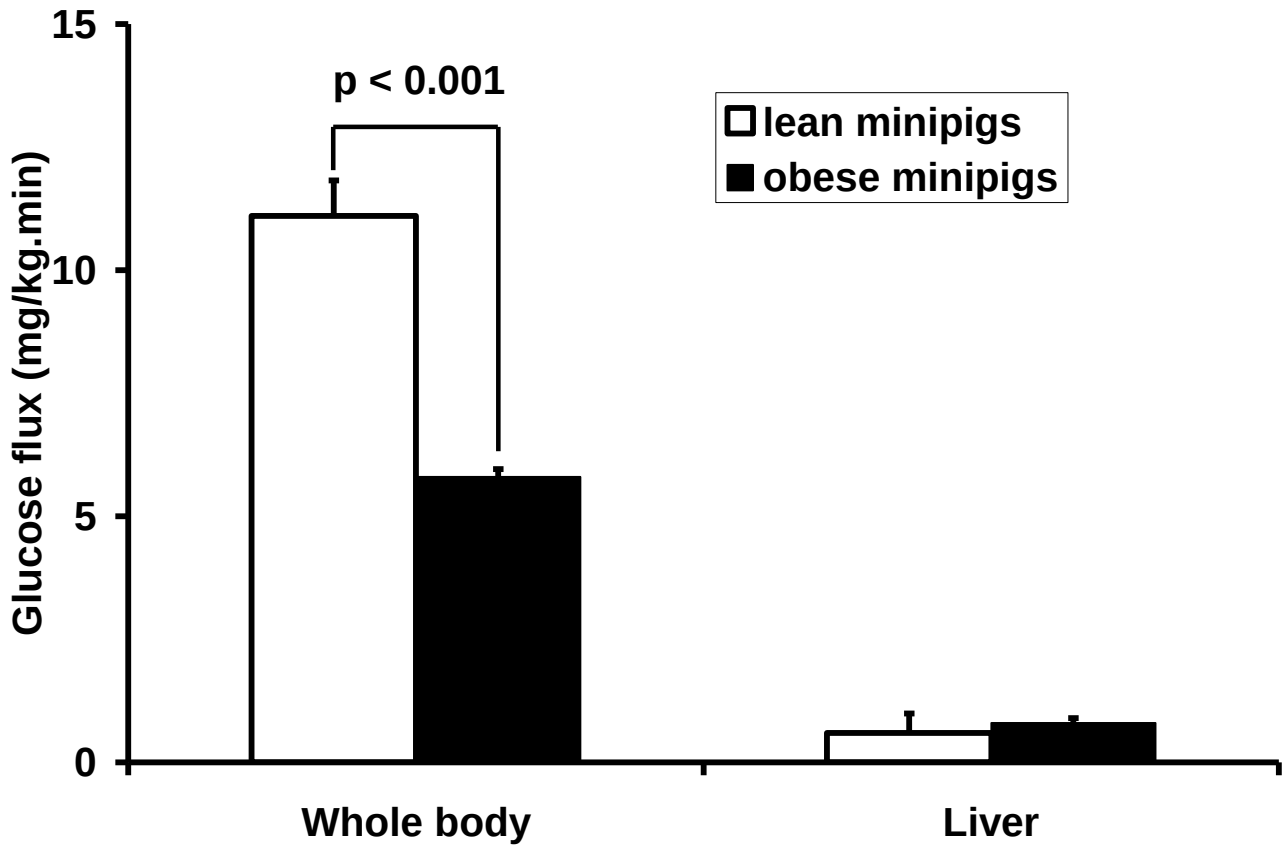
Insulin resistance (euglycemic clamp) in adult obese Göttingen minipigs on an obesogenic diet.



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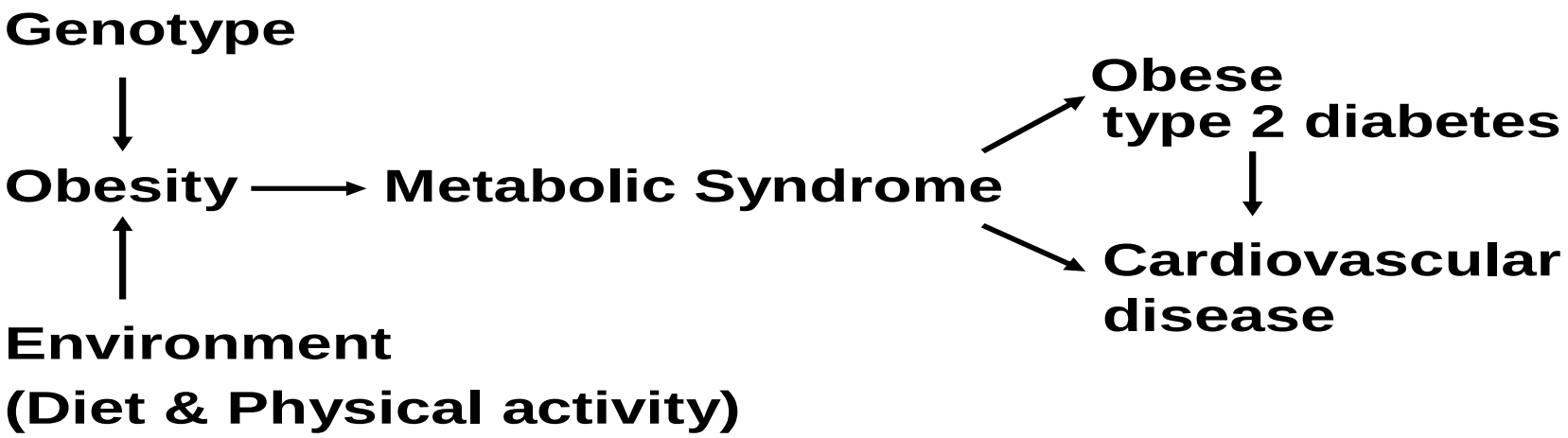
Development of metabolic and cardiovascular diseases



Chemical acceleration of diabetes

- Adult obese Göttingen minipigs (~80 kg) treated with multiple low doses (10-30 mg/kg) of streptozotocin (induces a further impairment of the functionality of insulin-producing pancreatic beta-cells) show a transition from the “metabolic syndrome phenotype” towards the “obese diabetic phenotype”.

Strategy 2: daily repeated streptozotocin (1 gram/day) until reaching 10 mmol/L blood glucose.

Method:

- Overnight fasting and day-time food availability after streptozotocin injection.
- Obese (~80 kg) female Göttingen Minipigs required 3 to 6 intravenous (ear vein) injections of streptozotocin to reach fasting blood glucose of 10 mmol/L.
- No short-term side-effects (complications) were observed like reduced food intake, vomiting or shivering.
- When fed 500 grams of mild obesogenic diet per day, body weight remained “stable”

Mild obesogenic diet

	Mild obesogenic diet					
	Ingredient %				Composition per kg	
Barley		10		Dry matter	g	926
Wheat		8		Crude ash	g	59
Soja hulls RC 320-360		36		Crude protein	g	130
Potato protein		4		Crude fat	g	138
Wheat gluten protein		5		Crude fiber	g	128
Sucrose		20		Starch (amylase method)	g	102
Lard		13		Sugars	g	223
Cholesterol		0.5		Non-starch polysaccharides	g	280
Limestone,CaCO3 (powder)		1.4		Gross energy diet	MJ	19
Mono-Calcium phosphate		1.8		Net energy diet	MJ	10
NaCl		0.6				
Premix 2 g/kg		0.2				
L-Tryptophan		0.01				
Total		**100.00**				

Blood and plasma analyses:

- In fresh heparin blood by venapuncture:

Glucose, ketones, cholesterol, HDL and triglyceride concentrations were measured on hand-held devices (blood glucose and ketone meter (On Call extra), ACON Laboratories, San Diego, USA; with ACON blood glucose and ketone test strips; and Mission Cholesterol Meter, ACON Laboratories, San Diego, USA; with 3-in-1 blood test strips).

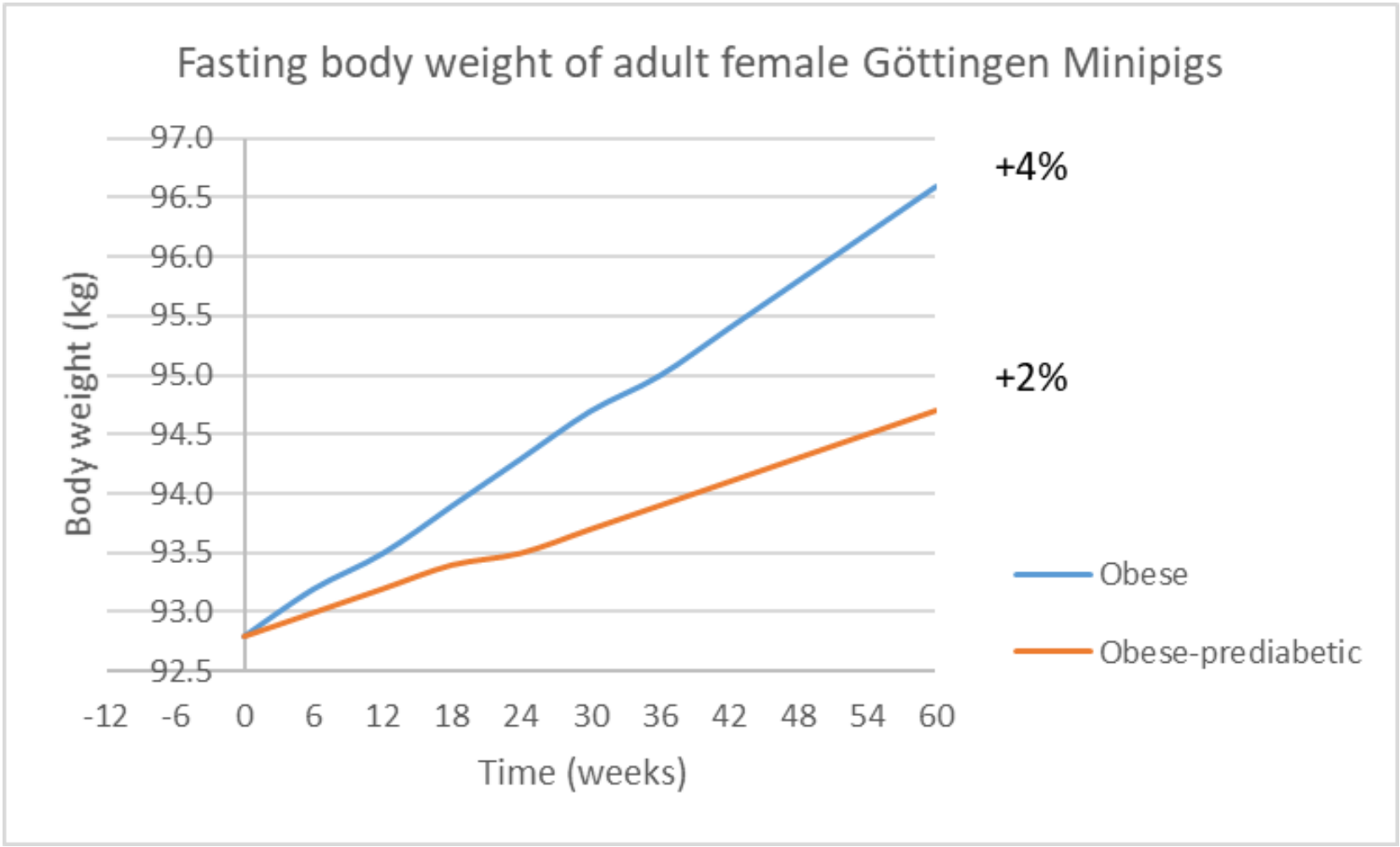
- In EDTA and heparin plasma:

Insulin concentration was measured using a porcine insulin ELISA kit (Mercodia, Uppsala, Sweden). Fructosamine concentration was measured using a nitroblue tetrazolium colometric assay (Abcam, Cambridge, United Kingdom).

Note: Glucose and metabolite concentrations can be validated by COBAS (Cobas C111, Analytical unit, Roche Diagnostics, Rotkreuz, Switzerland).

Obese-prediabetic: stability compared to obese.

Data normalized to Time=0, 500 g obesogenic diet/day



Obese-prediabetic: stability compared to obese.

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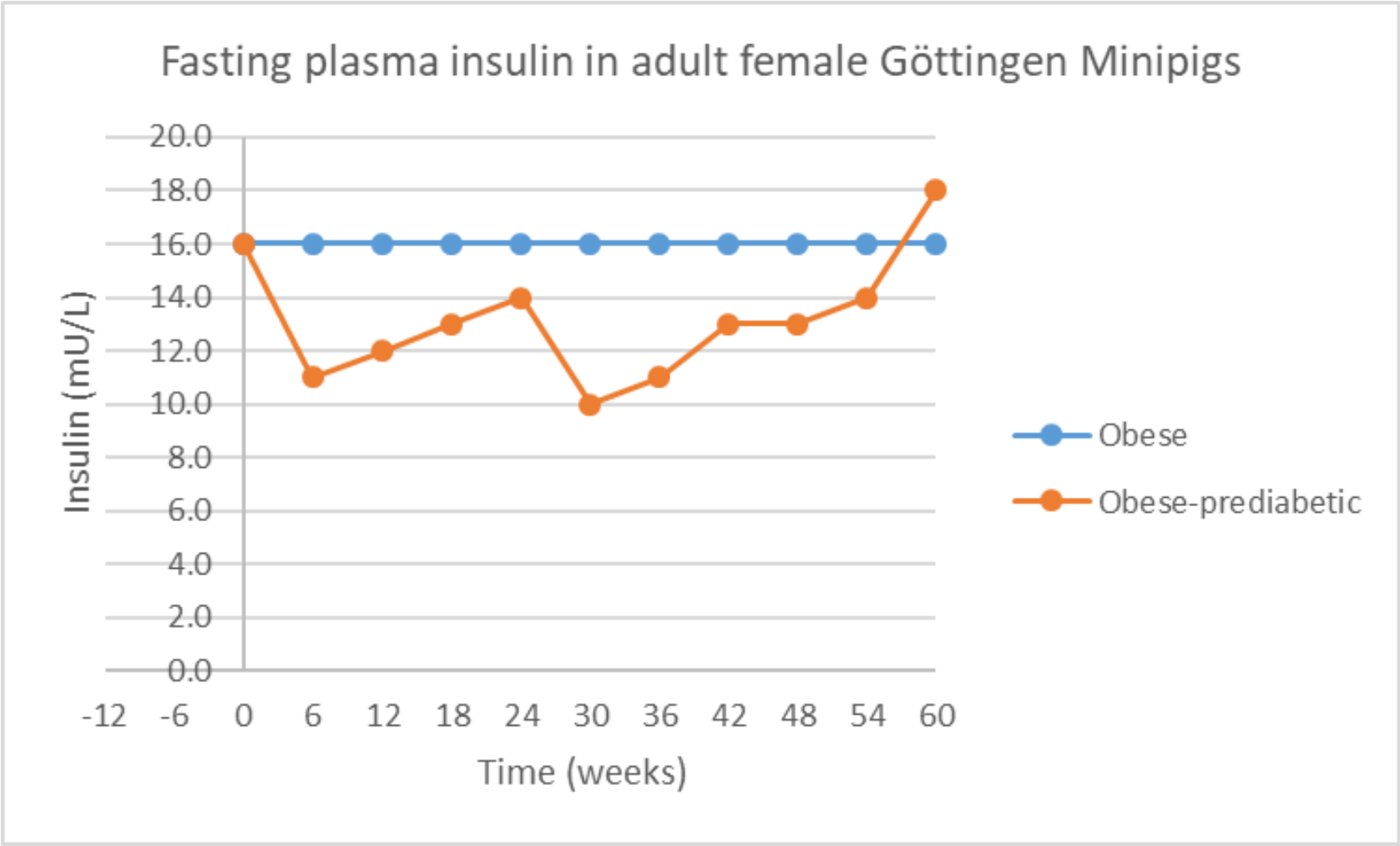

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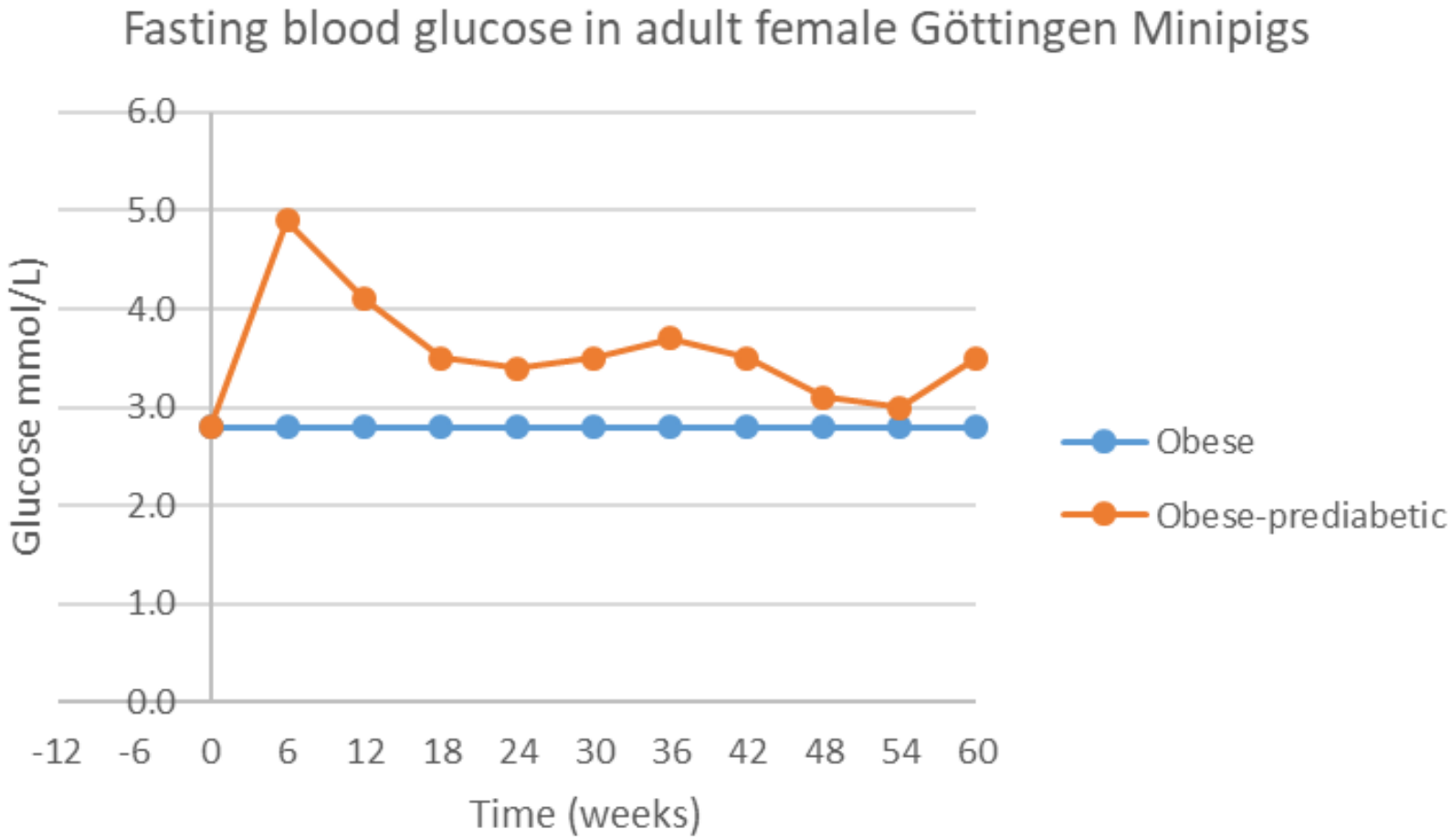

Obese-prediabetic: stability compared to obese.

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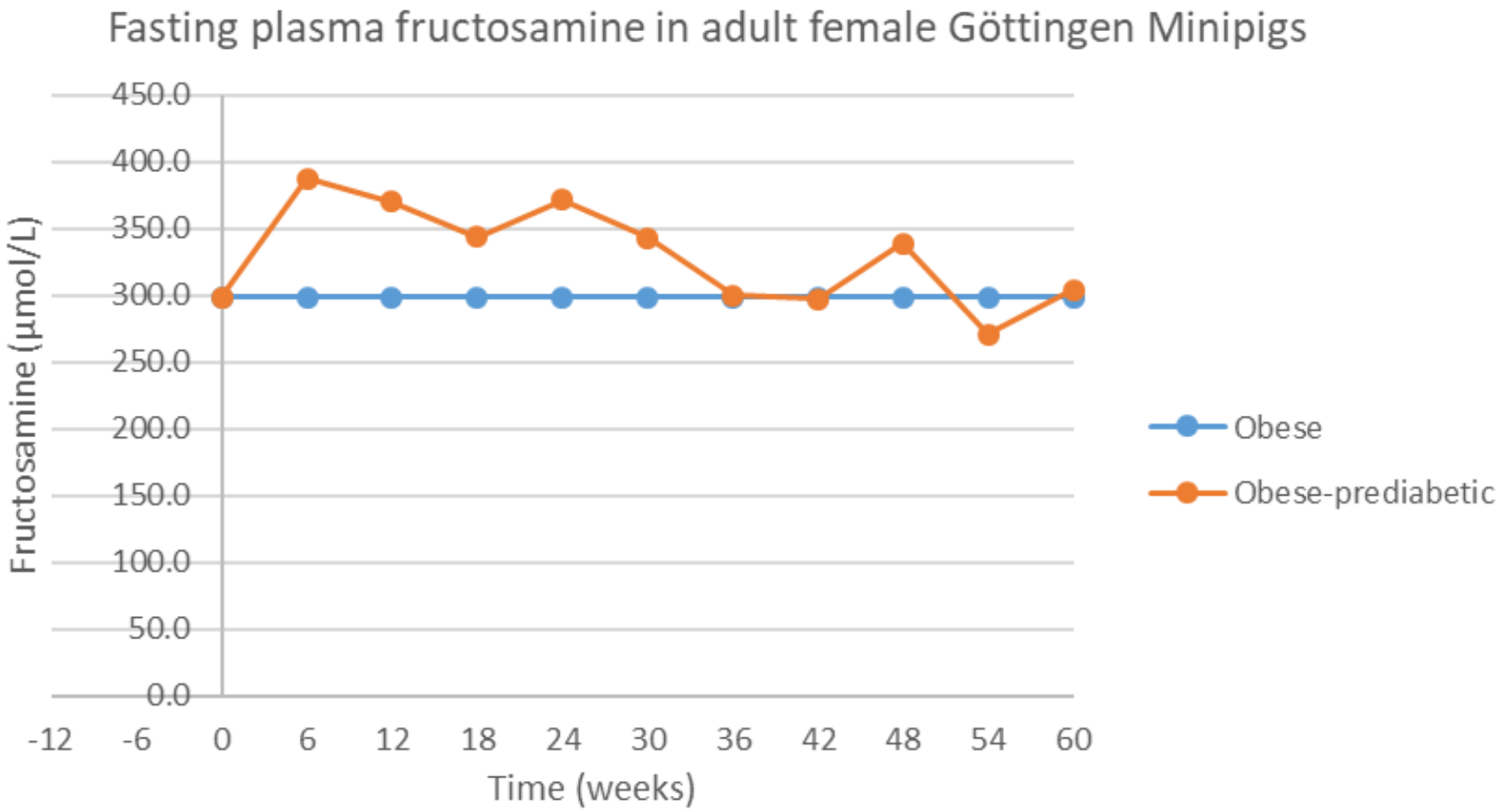

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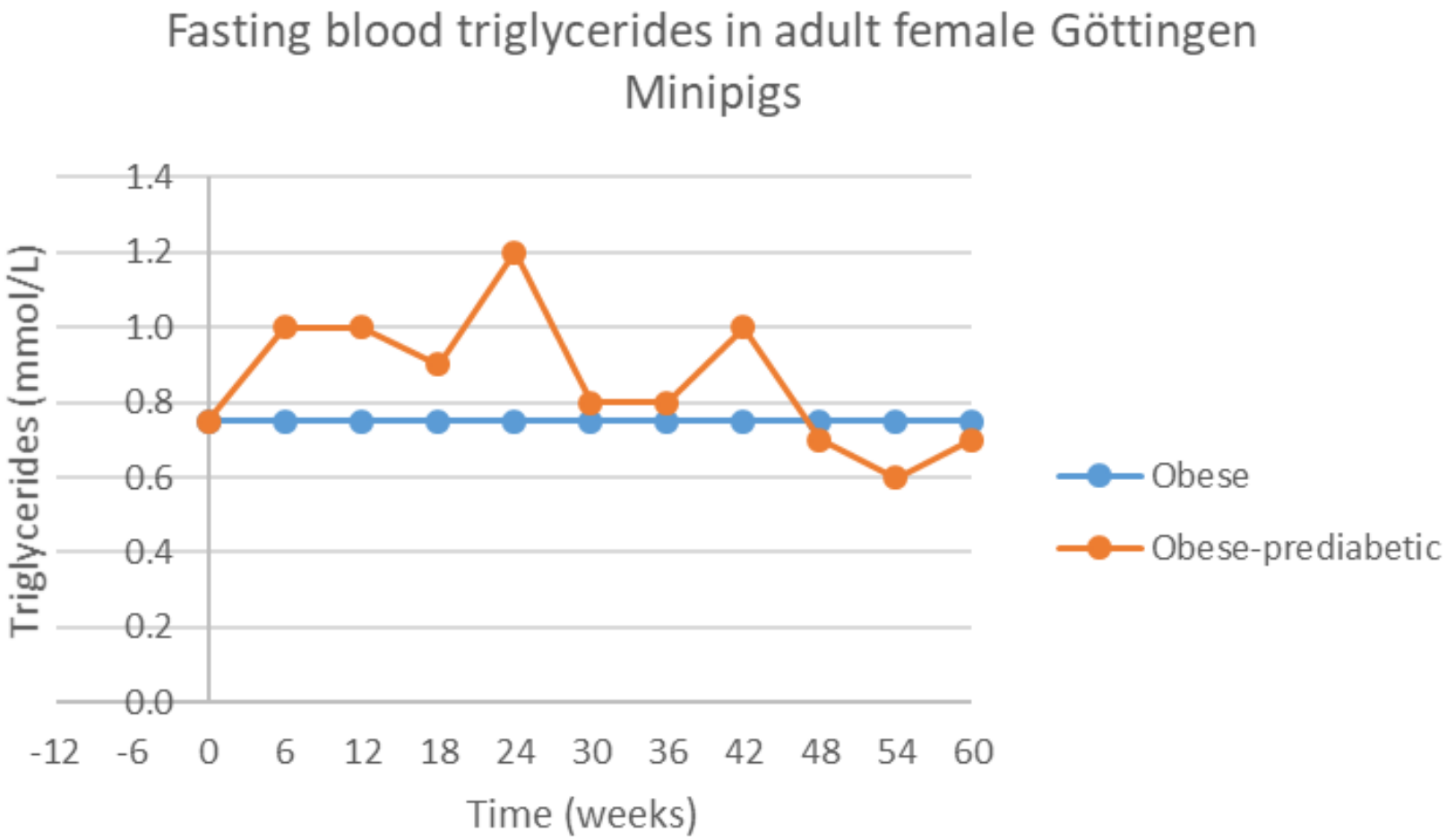

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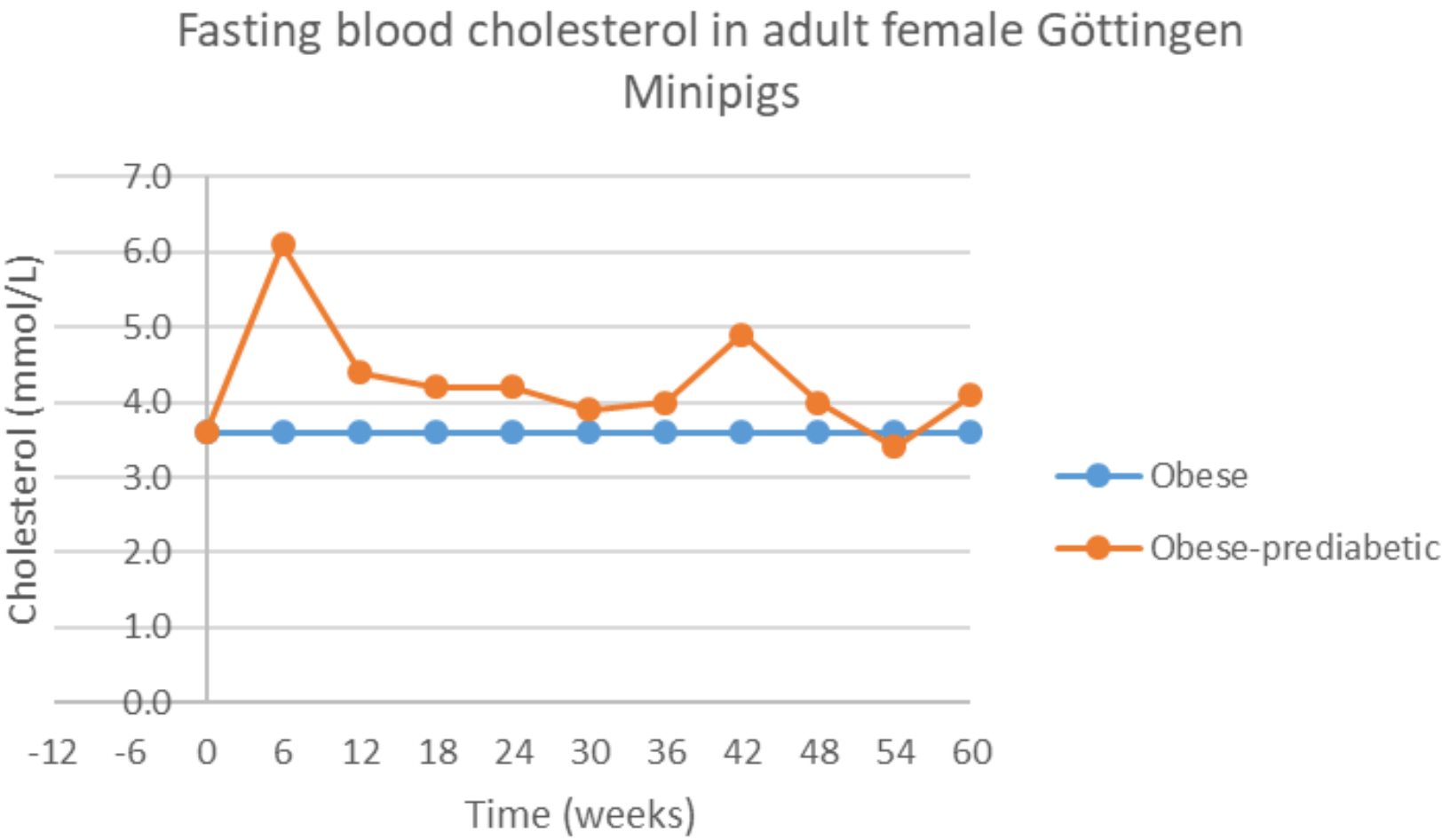

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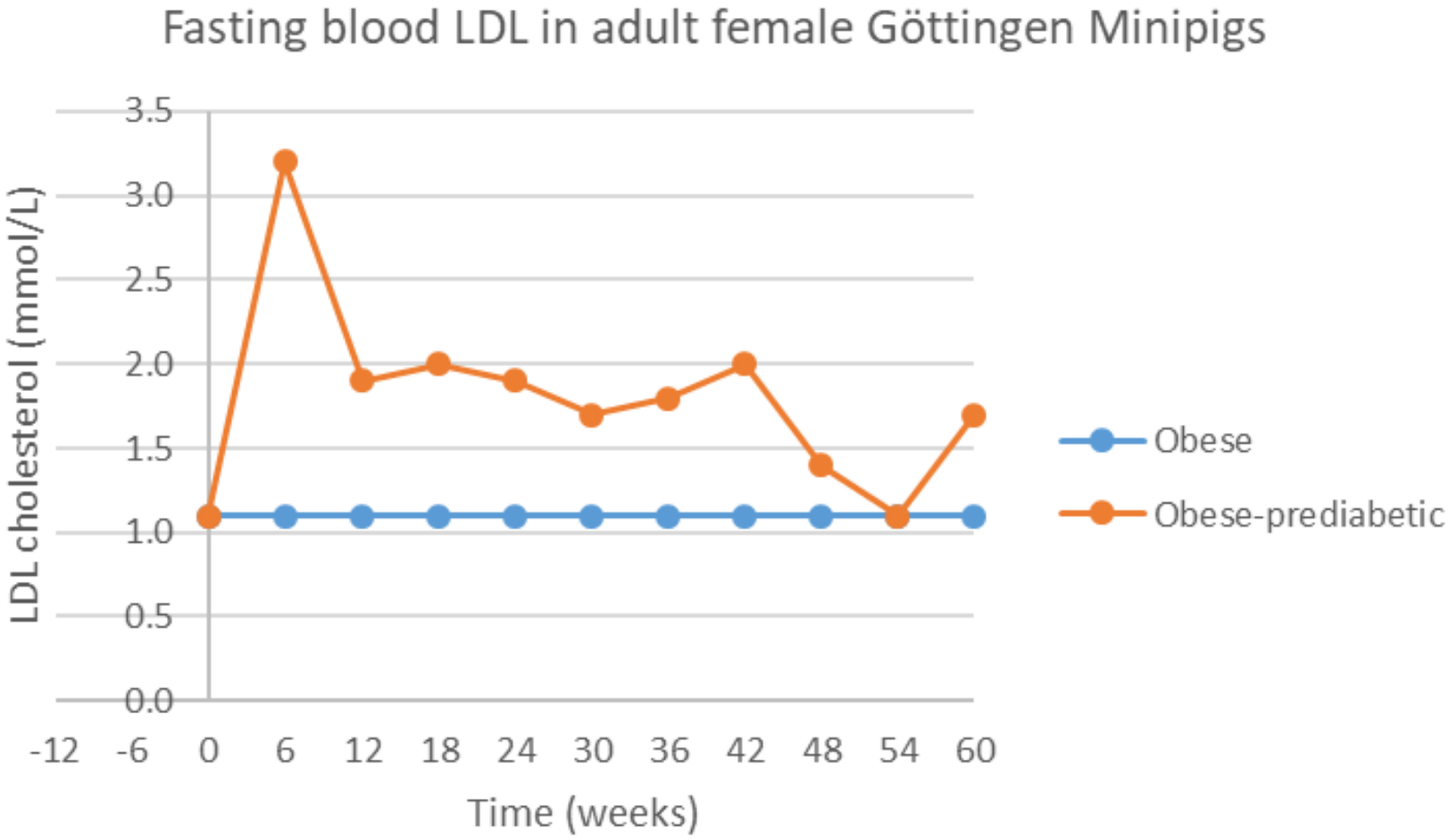

Obese-prediabetic: stability compared to obese.

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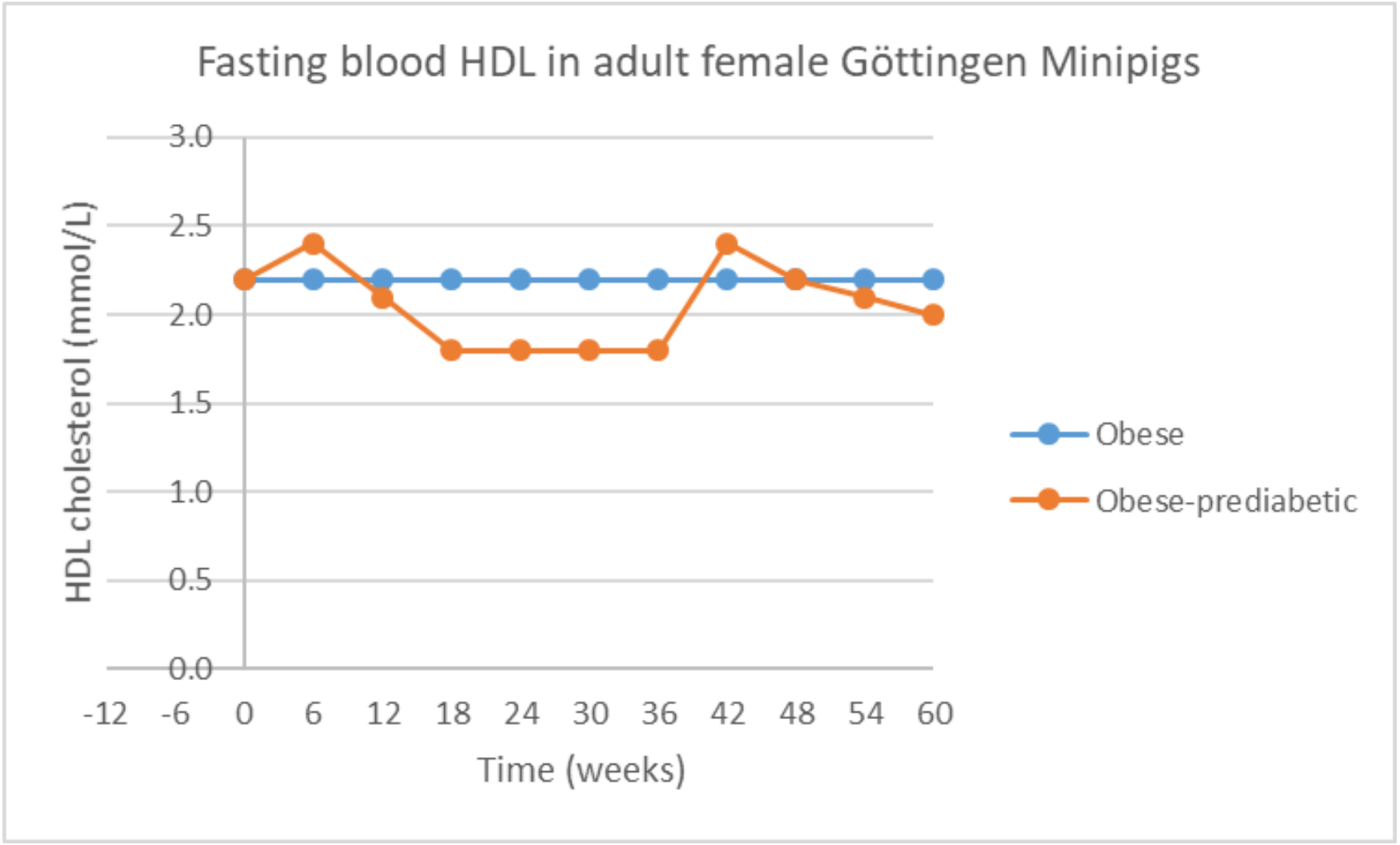

Obese-prediabetic: stability compared to obese.

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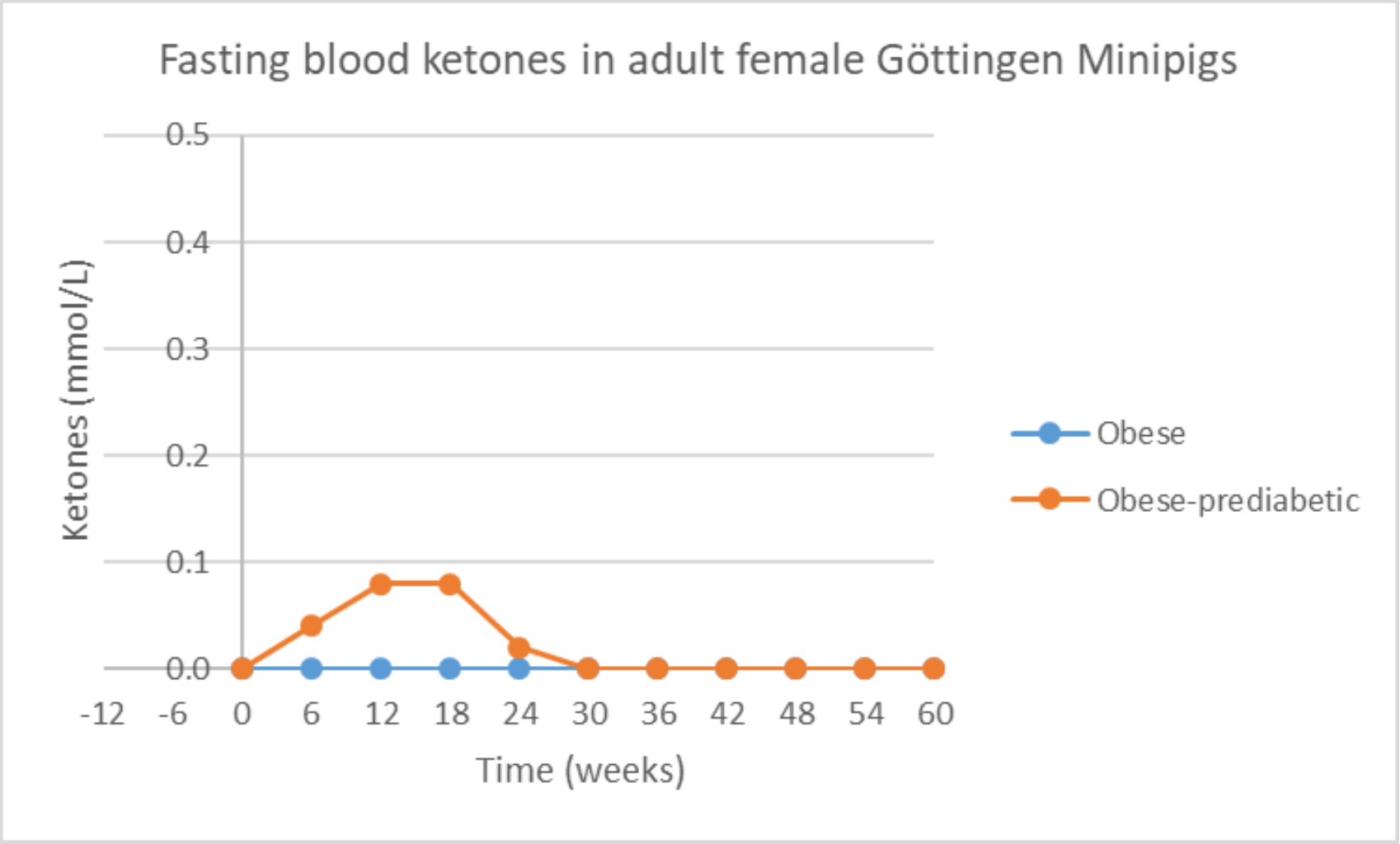
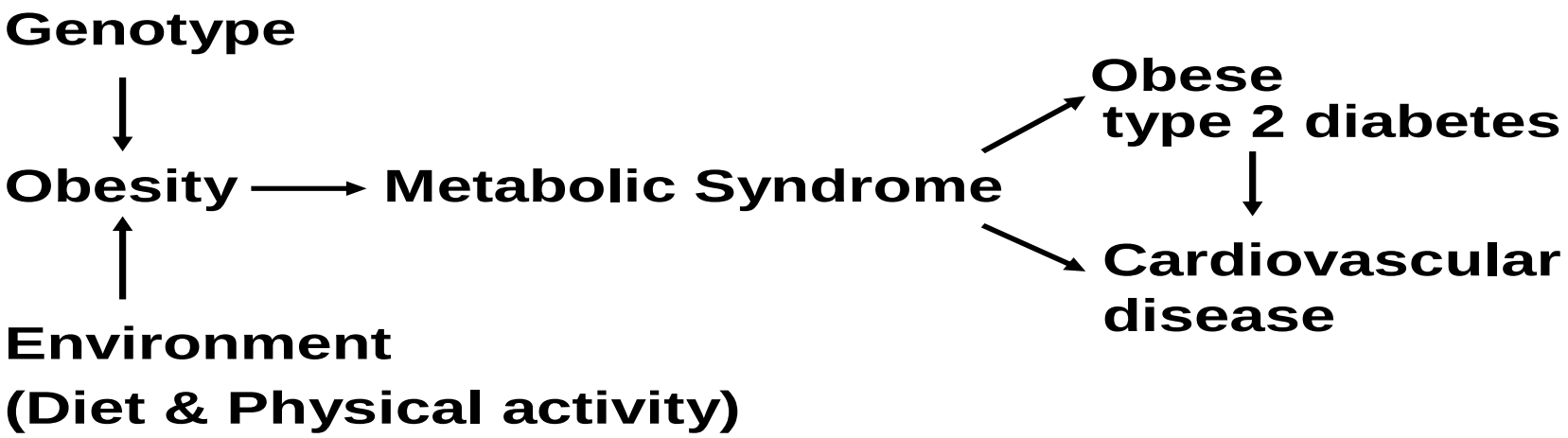


Strategy 2: daily repeated streptozotocin (1 gram/day) until reaching 10 mmol/L blood glucose.

Results and conclusions:

- Initial induction of diabetes which slowly (~6 months) returns back to a prediabetic phenotype.
- Time-course of recovery depends on measured blood parameter
- Most likely the pancreatic beta-cell mass is reduced substantially (to be measured) but sufficient to maintain metabolic homeostasis under the present experimental conditions.
- Outcome: obese prediabetic phenotype. When challenged, this pig model may become diabetic again.

Development of metabolic and cardiovascular diseases. Strategy 2: prediabetes



Strategy 3: two doses streptozotocin (1.5 grams first day and 3 grams second day. Blood glucose >10 mmol/L.

Method:

- Overnight fasting and day-time food availability after streptozotocin injection.
- Obese (~80 kg) female Göttingen Minipigs reached fasting blood glucose of 15-20 mmol/L after intravenous (ear vein) injections of streptozotocin.
- Short-term side-effects (complications) were observed like reduced food intake, vomiting or shivering and in 4 out of 9 pigs a long-term reduction in food intake was observed.

Strategy 3: two doses streptozotocin (1.5 grams first day and 3 grams second day. Blood glucose >10 mmol/L.

Warning!:

- Strategy 3 leads in ~half of the adult obese pigs in a long-term reduction in food intake.
- The mild obesogenic (high caloric) diet will not be consumed. Some pigs are willing to consume a low caloric, high fibre diet instead (i.e. the SDS-minipig diet) and other pigs are only willing to consume straw (fibre).
- At tissue collection no macroscopic abnormalities were observed in the gastrointestinal tract or other organs. Appetite-satiety mechanism affected?

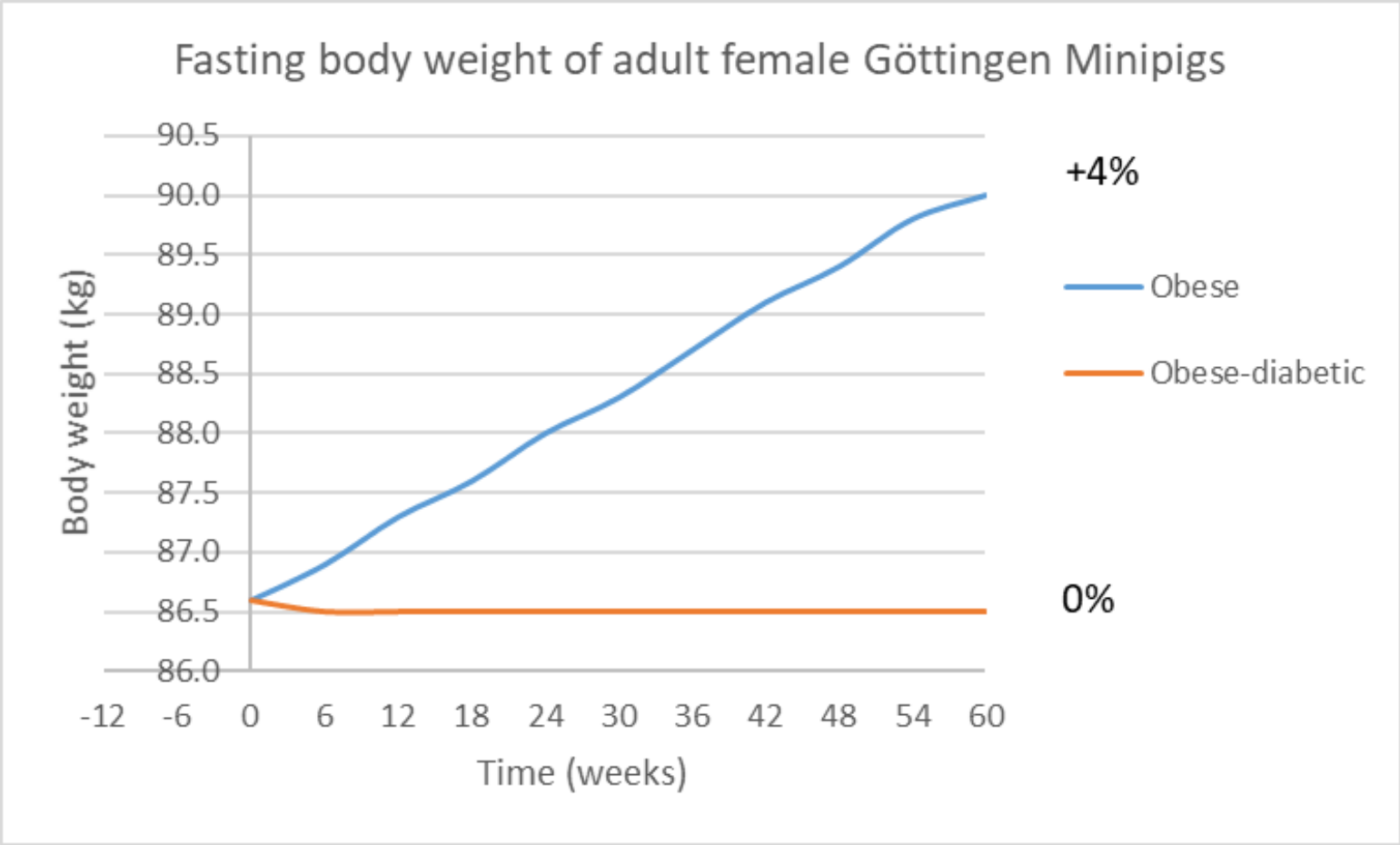
Strategy 4: two doses streptozotocin (1 gram first day and 2 grams second day. Blood glucose >10 mmol/L.

Method:

- Overnight fasting and day-time food availability after streptozotocin injection.
- Obese (~80 kg) female Göttingen Minipigs reached fasting blood glucose of ~15 mmol/L after intravenous (ear vein) injections of streptozotocin.
- Short-term side-effects (complications) were observed like reduced food intake, vomiting or shivering. No long-term reduction in food intake was observed. Alike a previous study (Coelho et al, J Oral Maxillofacial Surgery. 2018, 76:1677-87).

Obese-diabetic: stability compared to obese.

Data normalized to Time=0, 500 g obesogenic diet/day



Obese-diabetic: stability compared to obese.

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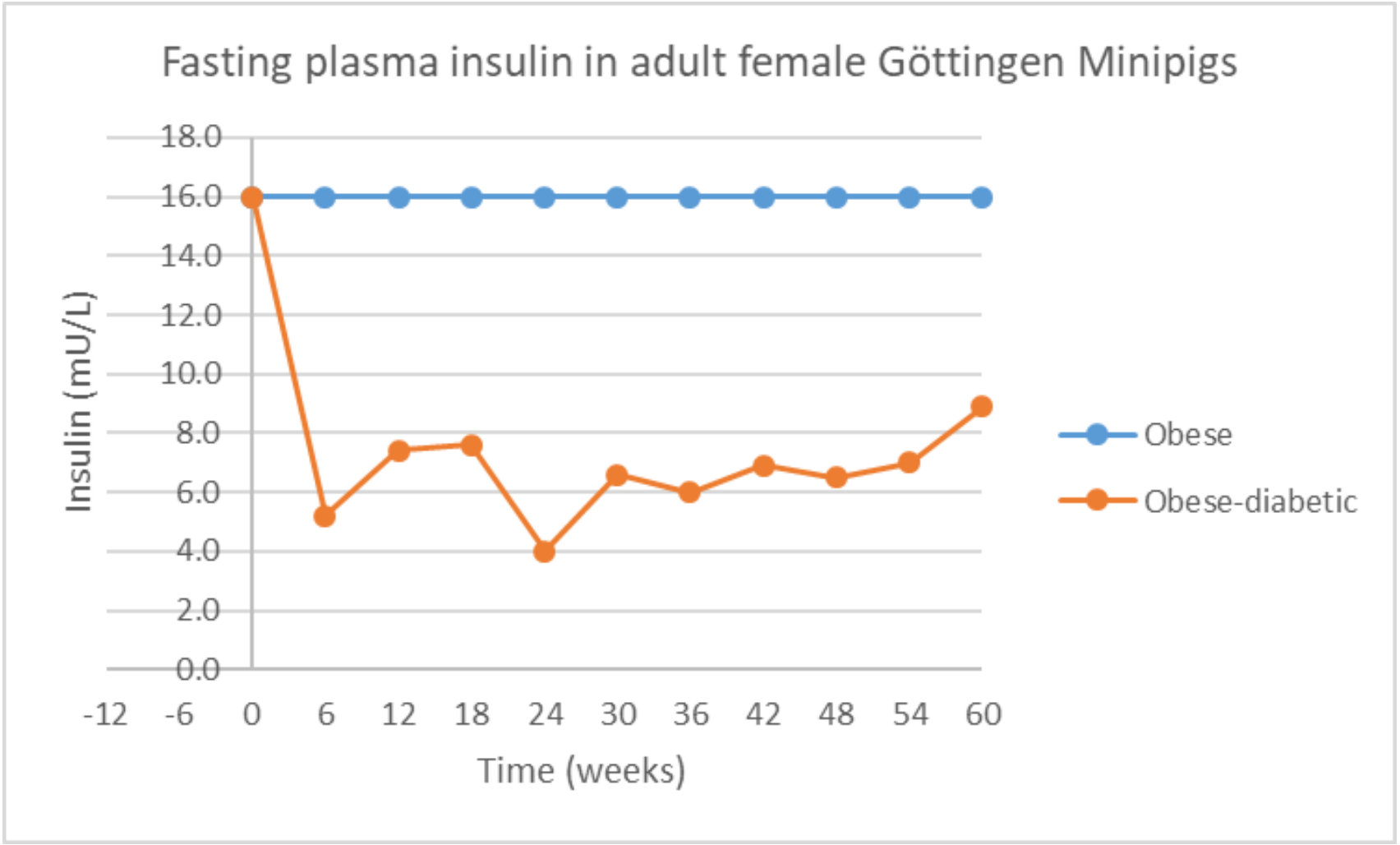

Obese-diabetic: stability compared to obese.

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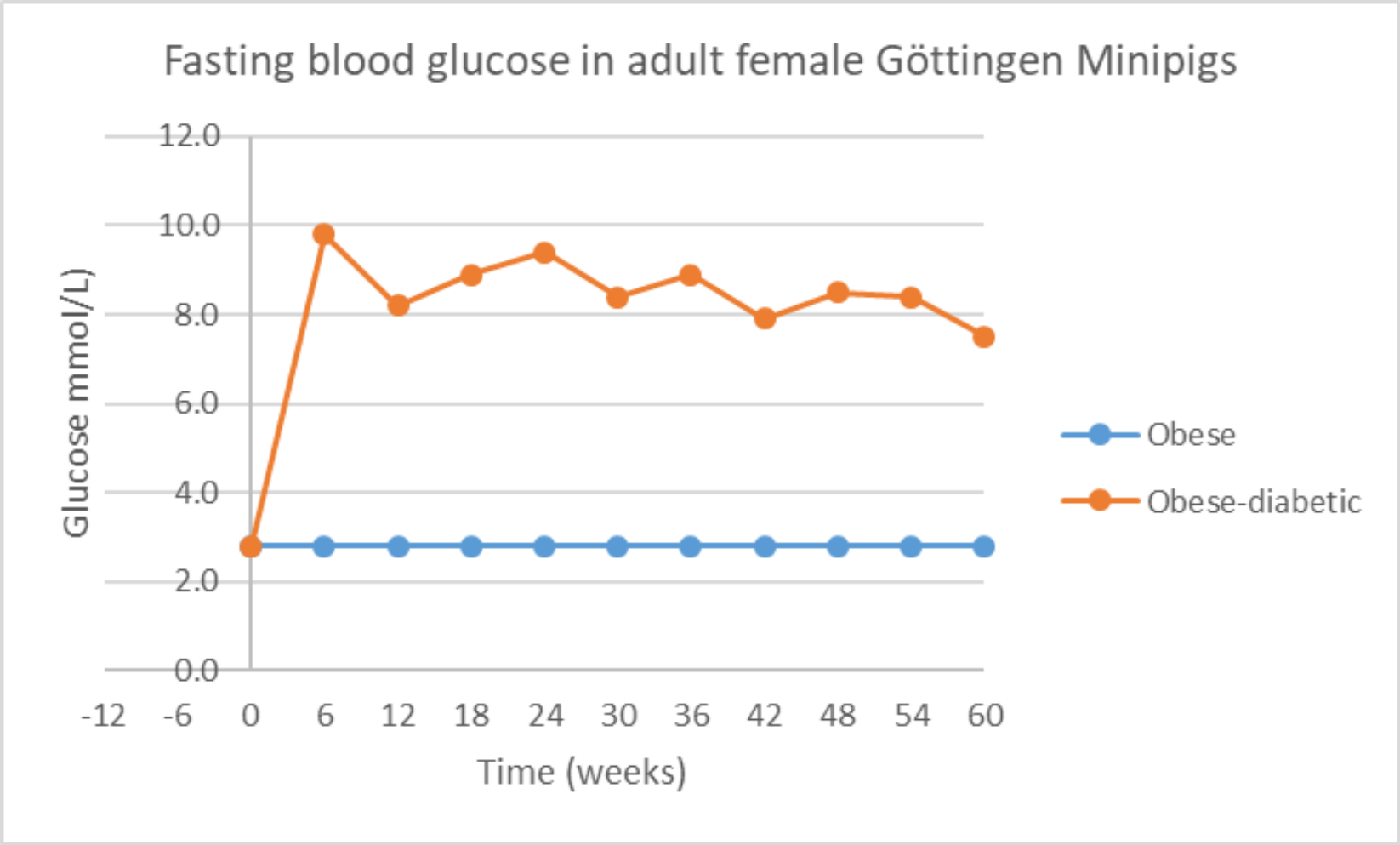

Obese-diabetic: stability compared to obese.

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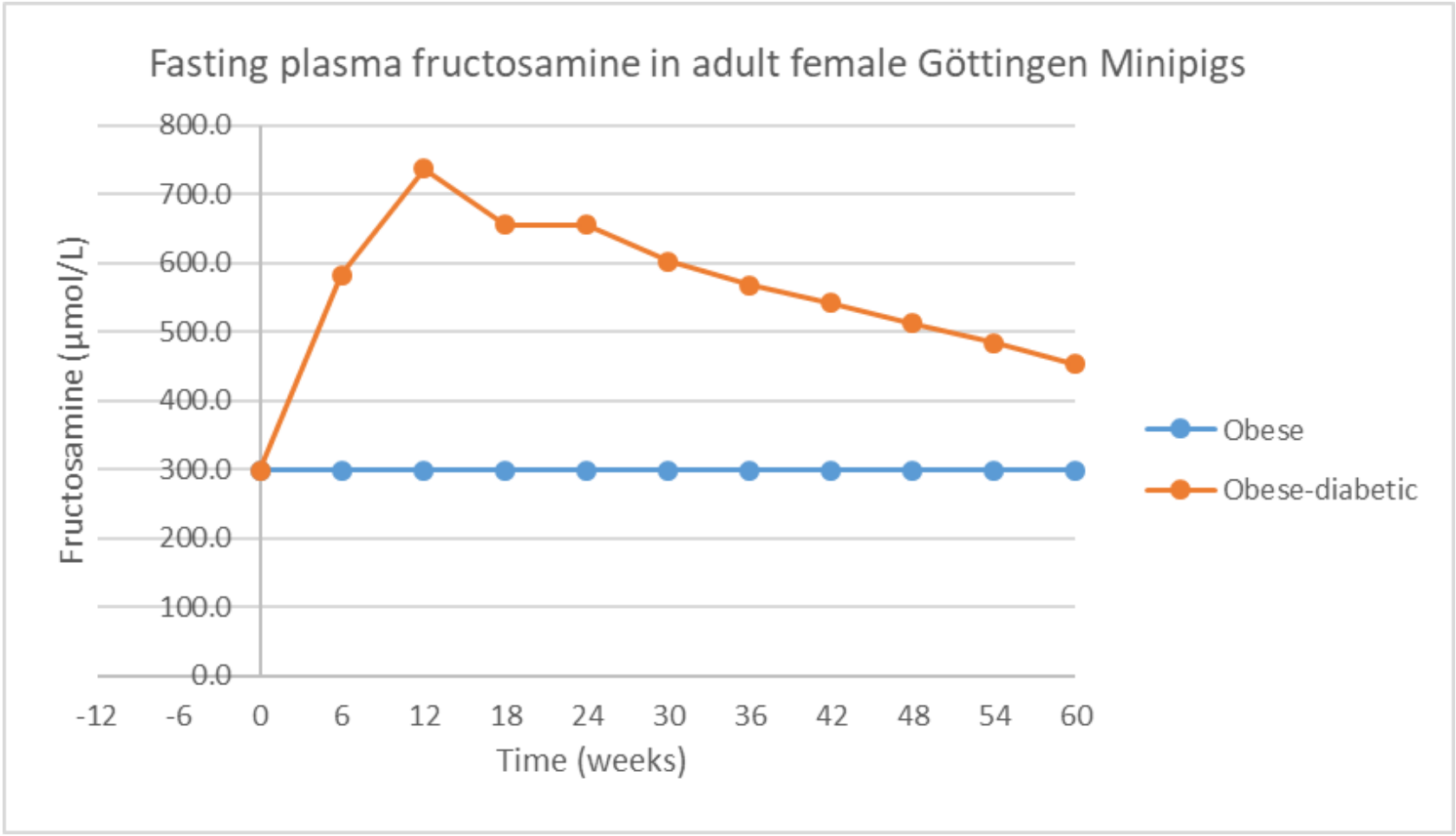

Obese-diabetic: stability compared to obese.

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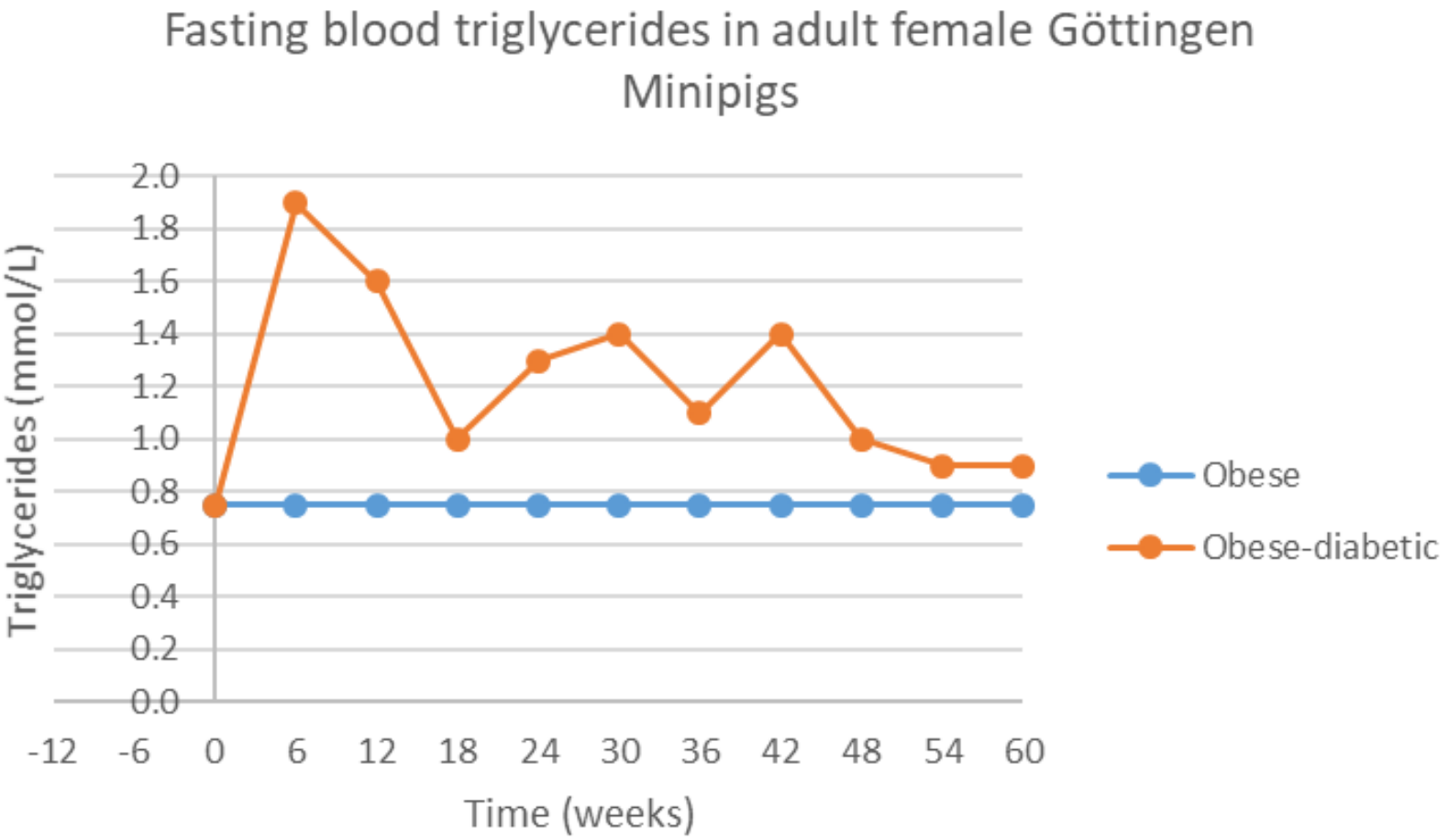

Obese-diabetic: stability compared to obese.

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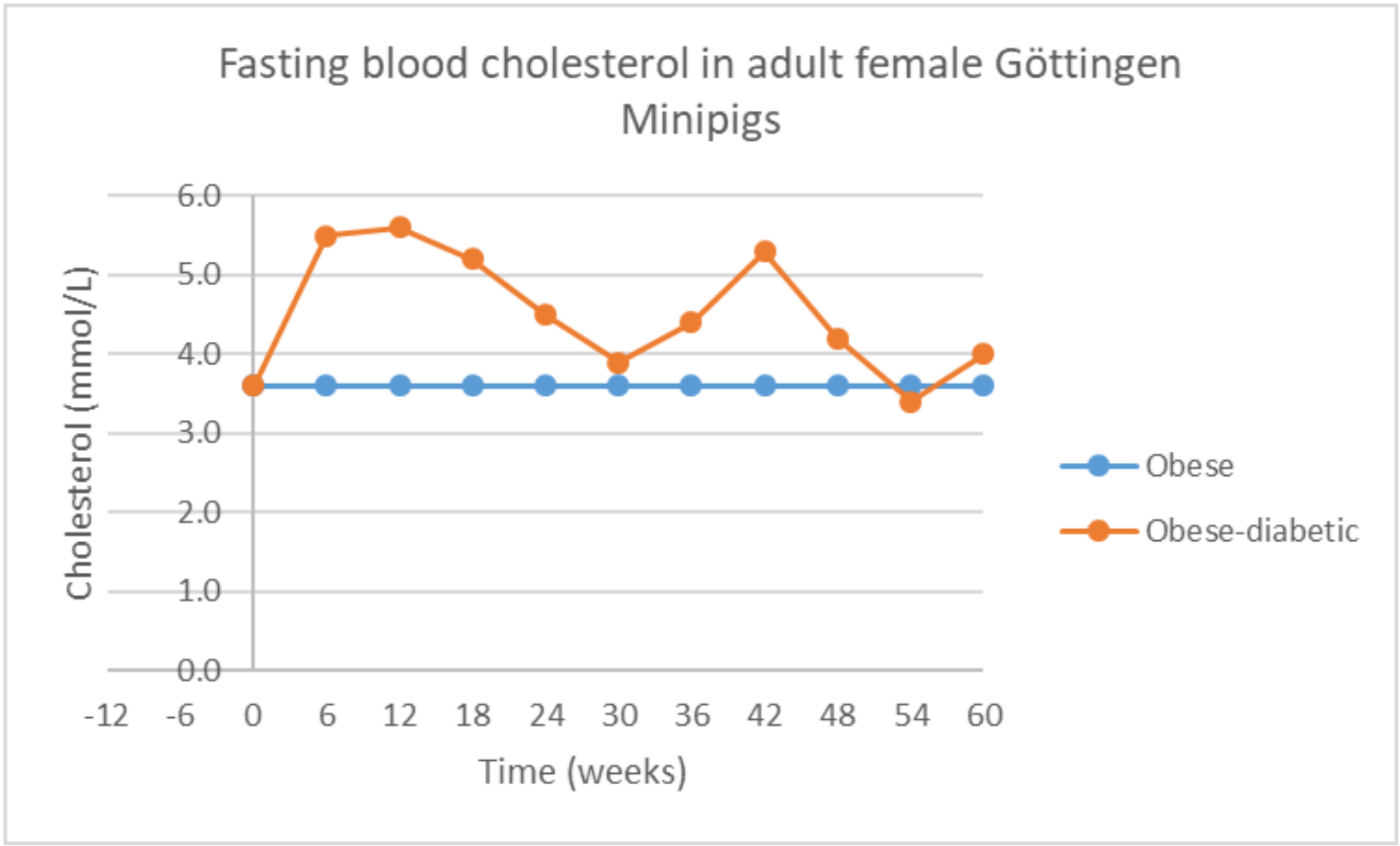

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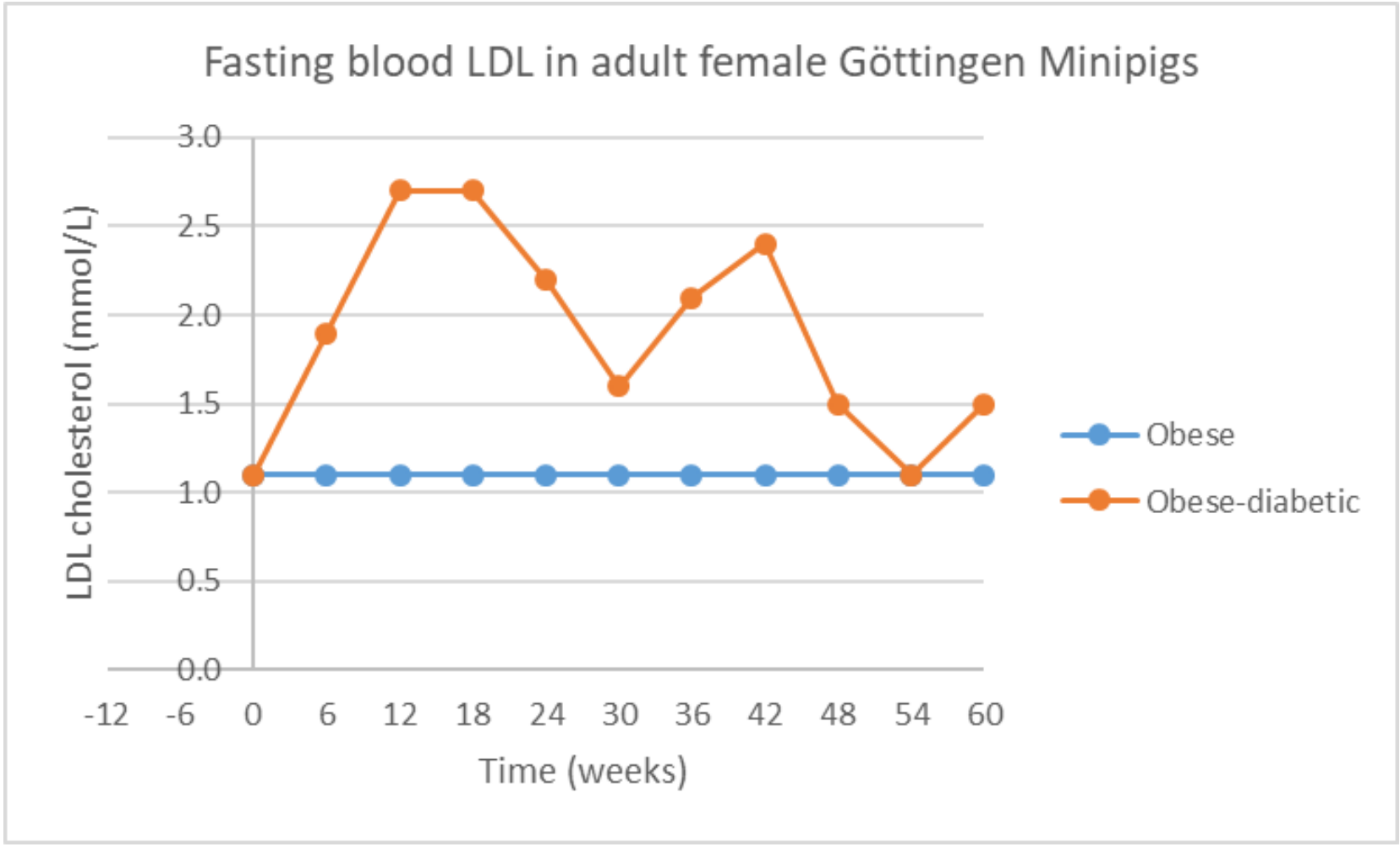

Obese-diabetic: stability compared to obese.

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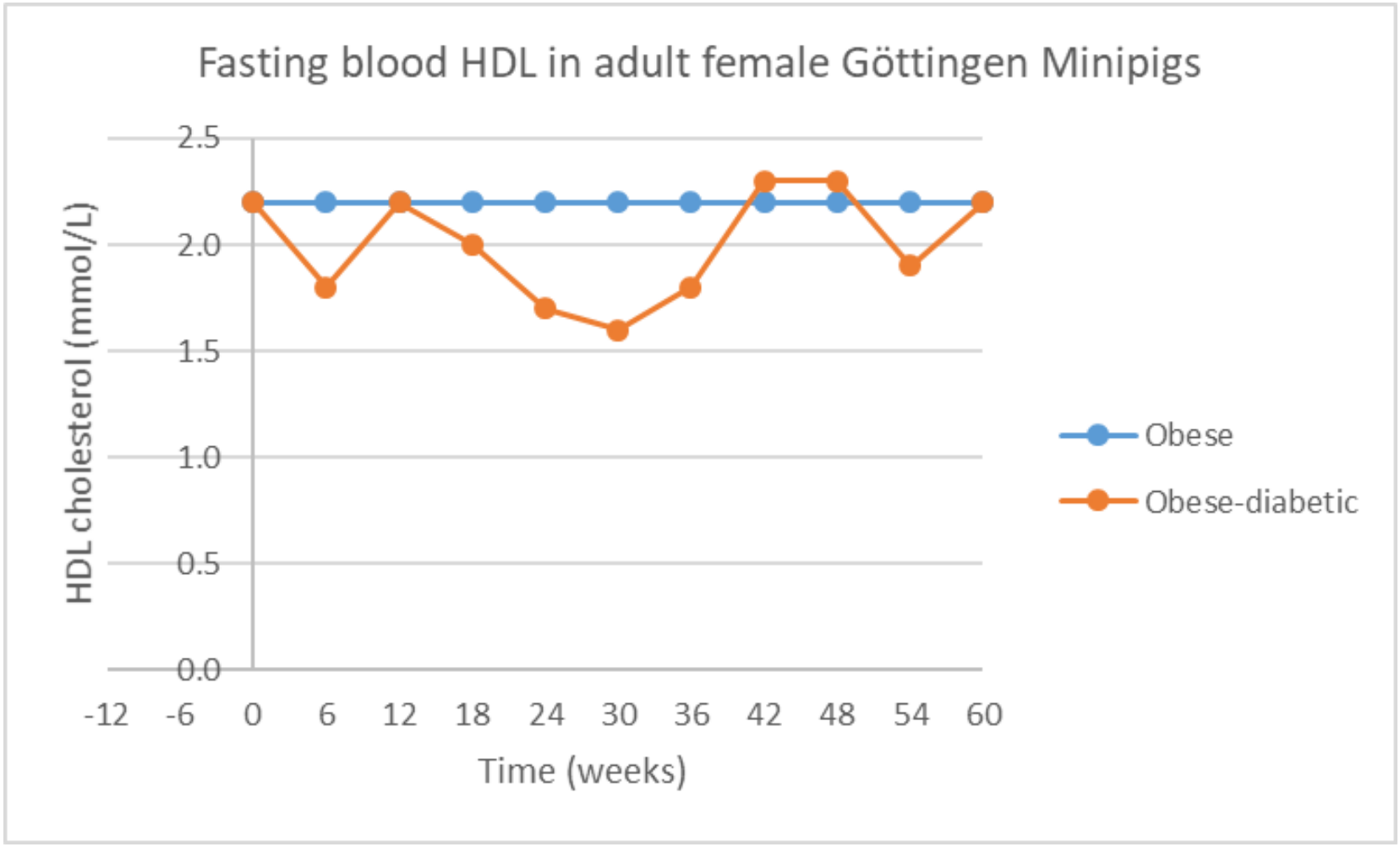

Obese-diabetic: stability compared to obese.

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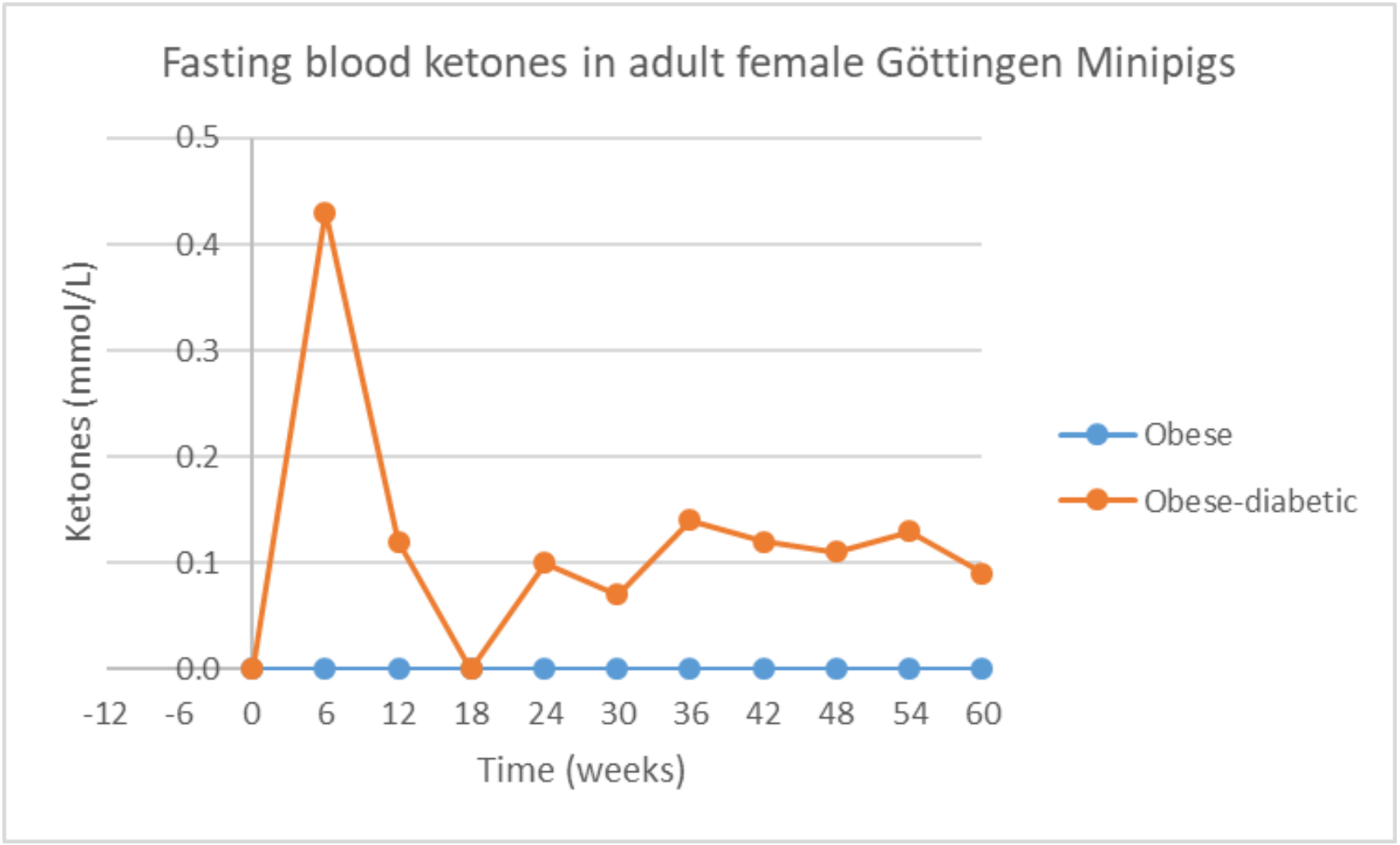
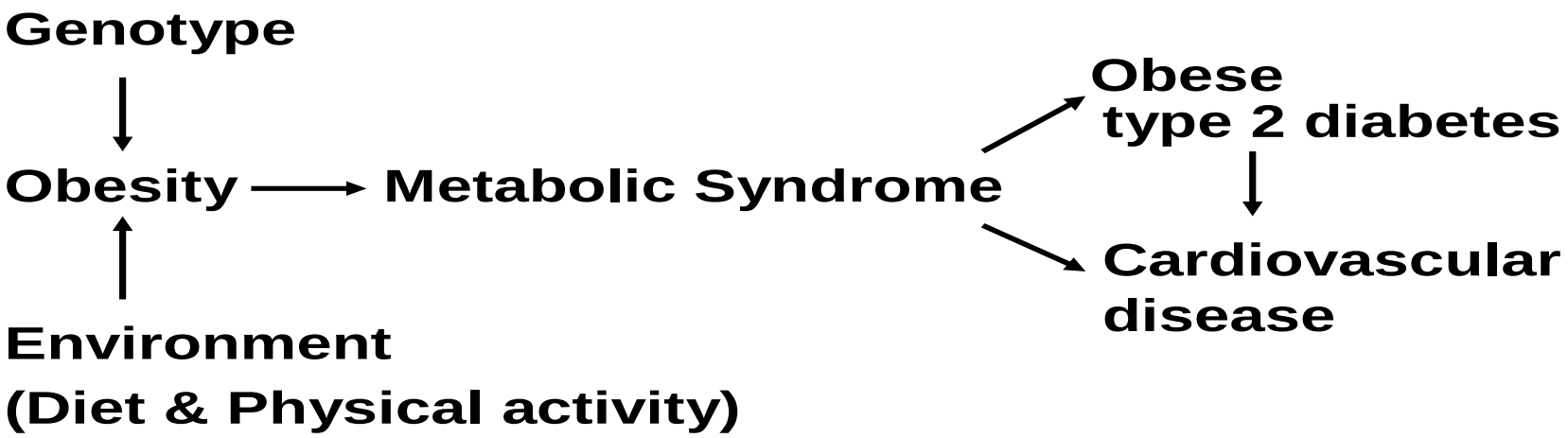


Strategy 3 and 4: two doses streptozotocin (1 or 1.5 gram first day and 2 or 3 grams second day). Blood glucose >10 mmol/L.

Results and conclusions:

- Induction of diabetes which slowly (~6-12 months) moves towards a more mild diabetic phenotype.
- Time-course of recovery depends on measured blood parameter
- Most likely the pancreatic beta-cell mass is reduced substantially (to be measured) and not sufficient to maintain metabolic homeostasis under the present experimental conditions.
- Outcome: obese diabetic phenotype.
- When fed 500 grams of mild obesogenic diet per day, body weight remained “stable”

Development of metabolic and cardiovascular diseases. Strategy 3 and 4: diabetes



Overall conclusion on the stability and usability of (pre)diabetic adult obese Göttingen Minipig models:

- Acute induction of (pre)diabetes in adult obese Göttingen minipigs by repeated intravenous administration of streptozotocin results in a (pre)diabetic phenotype which is fairly stable on the short-term (time period of several weeks) but evolves towards a more mild (pre)diabetic phenotype after 6 to 12 months. Pigs remain vital.
- For both short-term and long-term studies (i.e to test the effect of diet or medication) an appropriate time-control group of (pre)diabetic pigs needs to be included.